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(54) Title: CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES WHICH RECOGNIZE SURFACE PRO-  
TEINS FROM COAGULASE-NEGATIVE STAPHYLOCOCCI AND STAPHYLOCOCCUS AUREUS

(57) Abstract: Polyclonal and monoclonal antibodies which are cross-reactive to both coagulase-positive staphylococcus bacteria, such as *S. hemolyticus*, are provided which can recognize surface proteins from both coagulase-positive and coagulase negative staph bacteria. The antibodies may be generated from surface proteins that have been isolated on the basis of characteristics that may be common between *S. aureus* and coagulase-negative staphylococci, and these recombinant surface proteins are used to generate the antibodies of the invention. There is also provided vaccines and methods which utilize these proteins and antibodies for the treatment or protection against a wide variety of staphylococcal infections.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/19220

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01 N 33/569; C12 N 5/06, 5/16; C07 K 16/00

US CL : 435/7.33, 326, 332, 530/388.2, 388.4

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## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.33, 326, 332, 530/388.2, 388.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q9L470, 100% identical to SEQ.ID.NO: 21, SEQ.ID.NO: 19.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99QY4, 99.8% identical to SEQ.ID.NO: 18.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99QZ2, 97.4% identical to SEQ.ID.NO: 16.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99XE9, 92 % identical to SEQ.ID.NO: 12.	1-16, 19 and 21

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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## INTERNATIONAL SEARCH REPORT

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99UX5, 97.8 % identical to SEQ.ID.NO: 10.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99UX4, 98.8 % identical to SEQ.ID.NO: 8.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q931P4. 96.7 % identical to SEQ.ID.NO: 6 and Accession number Q99TD3, 96.6 % identical to SEQ.ID.NO: 6	1-16, 19 and 21
Y ✓	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99QY4, 98.6 % identical to SEQ.ID.NO: 4.	1-16, 19 and 21
Y	Database SPTREMBL, Swiss Institute for Bioinformatics, SIB ( Geneva, Switzerland), The European Bioinformatics Institute ,EBI (Cambridge, UK) Accession number Q99TB0, 91.6 % identical to SEQ.ID.NO: 2.	1-16, 19 and 21
Y	OHLSEN. K. et al Effects of subinhibitory concentrations of antibiotics on alpha-toxin (hla) gene expression of methicillin-sensitive and methicillin-resistant Staphylococcus aureus isolates. Antimicrob Agents Chemother, November 1998 , Vol 42, No. 11, pages 2817-2823.	1-16, 19 and 21

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/19220

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: Please See Continuation Sheet
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

PCT/US02/19220

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions 1-58 which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups 1-21 Claim(s) 1-14, 16, 19, 21 and 15, drawn to an isolated antibodies that bind to SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19, 21, nucleic acid sequence encoding amino acid sequences SEQ.ID.NOS: 1, 3, 5,7,9, 11, 13, 15, 20 and the nucleic sequences coding for the A domain of the Aap protein or degenerate.

Groups 22-33 Claims 20 and 22 drawn to fragment of the DsqA protein and a vaccine comprising a protein SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19 and 21

Groups 34-45 Claim 17 drawn to a method for treating or preventing S.aureus infection using antibodies that bind to SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19 and 21.

Groups 46-57 Claim 18 drawn to a method inducing an immune response using protein SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19 and 21.

The inventions listed as Groups 1-58 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group 1, claim(s) 1-14, 16, 19, 21 and 15, claim(s) 1-14, 16, 19, 21 drawn to an isolated antibodies that bind to SEQ.ID.NOS: 2, diagnostic kit comprising antibody to SEQ.ID.NOS: 2, pharmaceutical composition comprising said antibody and a method of diagnosing S.aureus infection using said antibody which is the first product and first product of use.

Pursuant to PCT Rule 13.2 the ISA/US considers that where multiple products, processes and methods are claimed, the main invention shall consists of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly the main invention (Group 1) comprises the first product and a method of use.

Further pursuant to PCT Rule 13.2 the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention. Therefore, the groups of inventions below do not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

Groups 2-21 drawn to different isolated antibodies that bind to SEQ.ID.NOS: 4, 6,8,10, 12, 14, 16, 17, 18, 19, 21, nucleic acid sequence encoding amino acid sequences SEQ.ID.NOS: 1, 3, 5,7,9, 11, 13, 15, 20 and the nucleic sequences coding for the A domain of the Aap protein or degenerate that are different to each other and lack the same or corresponding special technical features because each antibody bind to a protein having a specific amino acid sequence. They are structurally different to each other since each sequence has been identified with a specific sequence identification number that contains specific amino acids. In the instant case the different inventions represent structurally different antibodies that bind to different polypeptides. Therefore, where structural identity is required, such as for expression, the different sequences have different effects. Thus, each sequence is unique and lacks the same or corresponding special technical features.

Groups 22-33 drawn to fragment of the DsqA protein and a vaccine comprising a protein SEQ.ID.NOS: 2, 4, 6,8,10, 12, 14, 16, 17, 18, 19, and 21. These proteins are different to each other and lack the same or corresponding special technical features because each protein contains a specific amino acid sequence. They are structurally different to each other since each sequence has been identified with a specific sequence identification number that contains specific amino acids. In the instant case the different inventions represent structurally different proteins. Therefore, where structural identity is required, such as for expression, the different sequences have different effects. Thus, each sequence is unique and lacks the same or corresponding special technical features

## INTERNATIONAL SEARCH REPORT

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Groups 34-45 and 46-57 are different methods utilizing different products of antibodies or proteins that are unique and lack the same or corresponding special technical features that result in a different outcome such as preventing an infection with antibody or inducing an immune response with specific protein. These methods are different to each other in utilizing different reagents such as different polypeptides and antibodies as discussed above and thus lack the same or special technical features as explained above.

### Continuation of Box II Item 3:

1-16, 19 and 21 with respect to SEQ.ID.NOS: 2, 4, 6, 8, 10, 12, 16, 18, 19 and 21

### Continuation of B. FIELDS SEARCHED Item 3:

SEQ.ID.NOS: 2, 4, 6, 8, 10, 12, 14, 16, 19, 17, 18 and 21 searched on MEDLINE, STN, A -GENSEQ, N-GENSEQ, EST, DERWENT, SWISS-PROT, PIR, USPTOWEST, SWISSPTREMBL, GENEMBL, PUBLISHED APPLICATIONS AND ISSUED PATENTS

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(54) Title: CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES WHICH RECOGNIZE SURFACE PROTEINS FROM COAGULASE-NEGATIVE STAPHYLOCOCCI AND STAPHYLOCOCCUS AUREUS

(57) Abstract: Polyclonal and monoclonal antibodies which are cross-reactive to both coagulase-positive staphylococcus bacteria, such as *S. hemolyticus*, are provided which can recognize surface proteins from both coagulase-positive and coagulase negative staph bacteria. The antibodies may be generated from surface proteins that have been isolated on the basis of characteristics that may be common between *S. aureus* and coagulase-negative staphylococci, and these recombinant surface proteins are used to generate the antibodies of the invention. There is also provided vaccines and methods which utilize these proteins and antibodies for the treatment or protection against a wide variety of staphylococcal infections.



WO 02/102829 A2

**CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES  
WHICH RECOGNIZE SURFACE PROTEINS FROM COAGULASE-NEGATIVE  
STAPHYLOCOCCI AND STAPHYLOCOCCUS AUREUS**

**Cross Reference to Related Applications**

- 5           The present application claims the benefit of U.S. provisional application Ser. No. 60/298,098 filed June 15, 2001.

**Field of the Invention**

- 10           The present invention relates in general to surface proteins from *Staphylococcus aureus* and their active regions such as their A domains which have homologue proteins on coagulase-negative Staphylococci such as *S. epidermidis* and *S. hemolyticus* as well as antibodies which recognize said proteins, and in particular to isolated monoclonal and polyclonal antibodies which recognize specific proteins from *Staphylococcus aureus* and coagulase-negative Staphylococci and  
15           which are cross-reactive against *S. aureus* and coagulase-negative Staphylococci and can thus be utilized in vaccines and methods useful for preventing or treating a wide variety of infections caused by staphylococcal bacteria.

**Background of the Invention**

- 20           The successful colonization of the host is a process required for most microorganisms to cause infections in animals and humans. Microbial adhesion is the first crucial step in a series of events that can eventually lead to disease. Pathogenic microorganisms colonize the host by attaching to host tissues or serum conditioned implanted biomaterials, such as catheters, artificial joints, and vascular grafts, through specific adhesins present on the surface of the bacteria.  
25           MSCRAMM®s (Microbial Surface Components Recognizing Adhesive Matrix Molecules) are a family of cell surface adhesins that recognize and specifically bind to distinct components in the host's extracellular matrix. Once the bacteria have successfully adhered and colonized host tissues, their physiology is dramatically altered and damaging components such as toxins and proteolytic enzymes are  
30           secreted. Moreover, adherent bacteria often produce a biofilm and quickly become more resistant to the killing effect of most antibiotics.

*S. aureus* causes a spectrum of infections that range from cutaneous lesions such as wound infections, impetigo, and furuncles to life-threatening conditions that include pneumonia, septic arthritis, sepsis, endocarditis, and biomaterial related infections. *S. aureus* is known to express a repertoire of different MSCRAMMs that can act individually or in concert to facilitate microbial adhesion to specific host tissue components. In addition, another type of staphylococcus bacteria is identified as the coagulase-negative bacteria, including such species as *S. epidermidis* and *S. hemolyticus* which are also have been known to express MSCRAMMs, and which also are responsible for a wide range of bacterial infections and related diseases. In this regard, MSCRAMMs generally provide an excellent target for immunological attack by antibodies, both polyclonal and monoclonal antibodies.

However, because antibodies by nature are very specific and in the case of different types of Staphylococci, such as *S. aureus* on one hand (coagulase-positive) and *S. epidermidis* and *S. hemolyticus* on the other (coagulase-negative), it has still remained a significant problem to develop antibodies that exhibit cross-reactivity across the different types of bacteria. Such cross-reactive antibodies are particularly desirable because of their potential in immunizing human and animal patients and providing protection against infections caused by both types of Staphylococcal bacteria, namely coagulase-positive bacteria such as *S. aureus* and the coagulase-negative bacteria, such as *S. epidermidis* and *S. hemolyticus*. Such antibodies would thus be extremely useful in preventing or treating a wide variety of the infections caused by staphylococcal bacteria.

## Summary of the Invention

Accordingly, it is an object of the present invention to provide monoclonal antibodies that recognize MSCRAMM®s from both coagulase-positive bacteria such as *S. aureus* as well as MSCRAMM®s from coagulase-negative bacteria, such as *S. epidermidis* and *S. hemolyticus*.

It is also an object of the present invention to identify and isolate MSCRAMM®'s from staphylococcal bacteria, as well as their active regions such as the A domain, which can be used to generate monoclonal and polyclonal antibodies that will be cross-reactive against both coagulase-positive and coagulase-negative staphylococci.

It is still further an object of the present invention to provide isolated antibodies that can recognize the A domain of surface proteins such as the DgsK protein from coagulase-negative staphylococci and at the same time recognize surface proteins such as the SasA protein from *Staphylococcus aureus*.

It is yet another object of the present invention to utilize the isolated proteins, A domains and antibodies of the invention to produce vaccines useful in the treatment or prevention of staphylococcal infections, and to provide methods wherein the vaccines and antibodies of the invention are used to prevent or treat a staphylococcal infection.

These and other objects are provided by virtue of the present invention which comprises the identification and isolation of surface proteins from one type of staphylococcal bacteria, such as coagulase-negative or coagulase-positive staph, which can give rise to cross-reactive antibodies which can recognize surface proteins of both types of staph and which can thus be utilized in vaccines and methods of treating or preventing a wide range of staphylococcal infections. The present invention also relates to the generation of both polyclonal and monoclonal antibodies from these surface proteins and their use in preventing or treating staphylococcal infections.

These embodiments and other alternatives and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the present specification and/or the references cited herein, all of which are incorporated by reference.

#### Brief Description of the Drawing Figures

Figure 1 is a depiction of the primary structure of the *in silico*-predicted proteins in accordance with the present invention.

Figure 2 shows a Coomassie gel of the purified N-terminal recombinant His-tagged proteins expressing the orfs of the present invention.

5        Figures 3A-3C show Western blotting of *S. aureus* cell wall extracts showing probing with anti-KesK antibodies (Fig. 3A), anti-KnkA antibodies (Fig. 3B) and anti-DsqA antibodies (Fig. 3C), respectively.

10        Figures 4A-4B show Dot-blotting and Western immunoblotting of *Lactococcus lactis* expressing *S. aureus* MSCRAMM@s, namely KnkA (Fig. 4A) and KesK (Fig. 4B).

Figures 5A-5D representing the probing of recombinant LPXTG proteins in accordance with the present invention with convalescent sera examining *in vivo* expression, including RrKn and RrKN2 (Fig. 5A), KesK1 and KesK2A (Fig. 5B), KnkA (Fig. 5C) and DsqA2 (Fig. 5D).

15        Figure 6 shows a Western blot analysis demonstrating that rabbit polyclonal antibodies against *S. aureus* SasA cross-react with a protein released from the cell surface of *S. epidermidis* HB as well as the recombinant A-region from DsgK cloned from *S. epidermidis*.

## 20        DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the present invention, there are provided specific surface proteins from coagulase-positive staphylococcal bacteria, such as *S. aureus* as well as from coagulase-negative staph such as *S. epidermidis* and *S. hemolyticus*, including active fragments thereof such as the A domains of these proteins or other epitotic regions which can generate antibodies that recognize the whole protein. In accordance with the invention, the identification and isolation of candidate peptide sequences and proteins was carried out based on some of the common features of the MSCRAMM@s ((Microbial Surface Components Recognizing Adhesive Matrix Molecules) which are in most cases are covalently anchored to the cell wall

30        peptidoglycan. These surface proteins had the following common features which

were utilized in identifying and isolated the sequences of the present invention, namely: (i) an N-terminal signal peptide (approximately 40 residues in length) required for Sec-dependent secretion, (ii) a wall spanning domain either rich in proline and glycine residues or composed of serine and aspartate dipeptide repeats, (iii) an LPXTG motif required for covalent anchoring of the protein to the pentaglycine crossbridge in peptidoglycan, (iv) a hydrophobic membrane-spanning domain followed by (v) several positively charged residues.

In accordance with the invention, by exploiting the whole genome of *S. aureus* in light of the properties as set forth above, at least eight novel open reading frames encoding proteins with secretion and anchorage motifs indicative of MSCRAMMs were identified (i.e. bearing an N-terminal signal peptide and a C-terminal LPXTG motif followed by a hydrophobic domain and a positively charged tail). Table 1 illustrates the list of proteins identified including their distribution among *S. aureus* genomes, their protein size and C-terminal cell wall sorting sequence.

Table 1.

Name	Distribution	Size	C-terminus
EkeS	ENCSJM	2189 aa	LPNTGSEEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGGVMTLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK
KrkN2	ENCSJM (Cowan)	~278 aa	LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK
KrkN	ENCSJM	~661 aa	LPQTGEESNKDMTLPLMALIALSSIVAFVLP RKRKN
RkaS	ENCSJM	~801 aa	LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS
RrkN	NCSJM	1629 aa	LPKTGLESTQKGLIFSSIIGIAGLMLLARRRK N
KnkA	NCSJM	629 aa	LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKNKA

Abbreviations: eMRSA-16; N, 8325; C, COL; S, MSSA; J, N315, M, Mu50.

Six out of eight are conserved in all of the six staphylococcal genomes currently sequenced and the remaining two are present in 5/6 of these genomes.



In accordance with the invention, amino acid and nucleic acid sequences coding for the above proteins were obtained, and these were as follows: Ekes MRSA – SEQ ID NO:1 (DNA sequence); EkeS\_MRSA – SEQ ID NO:2 (Protein sequence); DsqA (8325) – SEQ ID NO:3 (DNA sequence); DsqA (8325) – SEQ ID NO:4 (Protein sequence); KesK1 (8325) – SEQ ID NO:5 (DNA sequence); KesK1 (8325) – SEQ ID NO:6 (Protein sequence); KrkN2 (8325) – SEQ ID NO:7 (DNA sequence); KrkN2 (8325) – SEQ ID NO:8 (Protein sequence); KrkN (8325) – SEQ ID NO:9 (DNA sequence); KrkN (8325) – SEQ ID NO:10 (Protein sequence); RkaS (COL) – SEQ ID NO:11 (DNA sequence); RkaS (COL) – SEQ ID NO:12 (Protein sequence); RrkN (8325) – SEQ ID NO:13 (DNA sequence); RrkN (8325) – SEQ ID NO:14 (Protein sequence); KnkA (8325) – SEQ ID NO:15 (DNA sequence); KnkA (8325) – SEQ ID NO:16 (Protein sequence).

In accordance with the present invention, isolated antibodies may be generated from the above proteins or their active regions such as the A domain so as to be able to recognize said proteins and/or said domains. These antibodies may be either monoclonal or polyclonal. If polyclonal antibodies are desired, these may be generated in any of a number of conventional ways well known in the art. In a typical process, the desired surface protein or active region thereof may be injected into a suitable host animal, e.g., a mouse or rabbit, and after a suitable time period, antibodies may be isolated and recovered from the host animal. With regard to monoclonal antibodies, in accordance with the present invention, these may be produced in any number of suitable ways including, e.g., the well known method of Kohler and Milstein, Nature 256:495-497 (1975), or other suitable ways known in the field, such as those methods disclosed in U.S. Pat. Nos. 6,331,415; 5,981,216; 5,807,715; and 4,816,567; Eur. Pat. App. 519,596; and PCT publication WO 00/71585, all of these patent publications incorporated herein by reference. These methods include their preparation as chimeric, humanized, or human monoclonal antibodies in ways that would be well known in this field. Still further, monoclonal antibodies may be prepared from a single chain, such as the light or heavy chains, and in addition may be prepared from active fragments of an

antibody which retain the binding characteristics (e.g., specificity and/or affinity) of the whole antibody. By active fragments is meant an antibody fragment which has the same binding specificity as a complete antibody which binds to the particular surface protein or its homologue from the different type of staph bacteria (i.e.,  
5 coagulase negative or coagulase-positive), and the term "antibody" as used herein is meant to include said fragments. Additionally, antisera prepared using monoclonal or polyclonal antibodies in accordance with the invention are also contemplated and may be prepared in a number of suitable ways as would be recognized by one skilled in the art.

10 As indicated above, antibodies to the isolated surface proteins and/or their active regions in accordance with the invention may be prepared in a number of suitable ways that would be well known in the art, such as the well-established Kohler and Milstein method described above which can be utilized to generate monoclonal antibodies. For example, in preliminary steps utilized in such a  
15 process, mice may be injected intraperitoneally once a week for a prolonged period with a purified recombinant MSCRAMM® in accordance with the invention or an active portion thereof, followed by a test of blood obtained from the immunized mice to determine reactivity to the purified protein. Following identification of mice reactive to the proteins, lymphocytes isolated from mouse spleens are fused to  
20 mouse myeloma cells to produce hybridomas positive for the antibodies against the surface proteins of the invention which are then isolated and cultured, following by purification and isotyping.

In order to generate monoclonal antibodies in accordance with the invention, it is preferred that these be generated using recombinantly prepared MSCRAMM®'s  
25 in accordance with the invention, and these recombinants may be generated and isolated using a number of standard methods well known in the art. For example, one such method employs the use of *E. coli* expression vector pQE-30 as an expression vector for cloning and expressing recombinant proteins and peptides. In one preferred method, using PCR, the A domain of the surface protein identified as  
30 DgsK or SasA was amplified from the sequences described above and subcloned

into the *E. coli* expression vector PQE-30 (Qiagen), which allows for the expression of a recombinant fusion protein containing six histidine residues. This vector was subsequently transformed into *E. coli* strain ATCC 55151, grown in a 15-liter fermentor to an optical density (OD<sub>600</sub>) of 0.7 and induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells were harvested using an AG Technologies hollow-fiber assembly (pore size 0.45 µm) and the cell paste frozen at -80° C. Cells were lysed in 1X PBS (10 mL buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi. Lysed cells were spun down at 17,000rpm for 30 minutes to remove cell debris. Supernatant was passed over a 5-mL HiTrap Chelating (Pharmacia) column charged with 0.1M NiCl<sub>2</sub>. After loading, the column was washed with 5 column volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200 mM imidazole (Buffer B) over 30 column volumes. SdrGN1N2N3 or SdrGN2N3 eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored. Fractions containing SdrGN1N2N3 or SdrGN2N3 were dialyzed in 1x PBS.

Next, each protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10% Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column, charged with 0.1M NiCl<sub>2</sub> to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reapplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The

purified product was analyzed for concentration, purity and endotoxin level before administration into the mice.

In the preferred process, monoclonal antibodies in accordance with the present invention may be prepared from the recombinant proteins identified above in the following manner. In this process, *E. coli* expressed and purified recombinant SasA and DsgK proteins were used to generate a panel of murine monoclonal antibodies while the mouse sera was used as a source of polyclonal antibodies. Briefly, a group of Balb/C or SJL mice received a series of subcutaneous immunizations of 1-10 mg of protein in solution or mixed with adjuvant as described below in Table 2.

**Table 2. Immunization Schemes**

RIMMS				
Injection	Day	Amount (µg)	Route	Adjuvant
#1	0	5	Subcutaneous	FCA/RIBI
#2	2	1	Subcutaneous	FCA/RIBI
#3	4	1	Subcutaneous	FCA/RIBI
#4	7	1	Subcutaneous	FCA/RIBI
#5	9	1	Subcutaneous	FCA/RIBI
Conventional				
Injection	Day	Amount (µg)	Route	Adjuvant
Primary	0	5	Subcutaneous	FCA
Boost #1	14	1	Intraperitoneal	RIBI
Boost #2	28	1	Intraperitoneal	RIBI
Boost #3	42	1	Intraperitoneal	RIBI

At the time of sacrifice (RIMMS) or seven days after a boost (conventional) serum was collected and titered in ELISA assays against MSCRAMM<sup>®</sup> proteins or on whole cells (*S. epidermidis* and *S. aureus*). Three days after the final boost, the spleens or lymph nodes were removed, teased into a single cell suspension and the lymphocytes harvested. Lymphocytes were then fused to a P3X63Ag8.653 myeloma cell line (ATCC #CRL-1580). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.), incorporated herein by reference.

Any clones that were generated from the fusion were then screened for specific anti-SasA antibody production using a standard ELISA assay. Positive clones were expanded and tested further for activity in a whole bacterial cell binding assay by flow cytometry and SasA binding by Biacore analysis. Throughout the Biacore analysis, the flow rate remained constant at 10 ml/min. Prior to the SasA or DgsK injection, test antibody was adsorbed to the chip via RAM-Fc binding. At time 0, SasA or DgsK at a concentration of 30 mg/ml was injected over the chip for 3 min followed by 2 minutes of dissociation. This phase of the analysis measured the relative association and disassociation kinetics of the Mab/SasA or DgsK interaction.

Next, the antibodies prepared as set forth above were tested for binding to whole bacteria. In these tests, bacterial samples *S. aureus* Newman, *S. aureus* 67-0, *S. aureus* 397 (Sal6), *S. aureus* Wood, *S. aureus* 8325-4, methicillin resistant *S. aureus* MRSA 16, *S. epidermidis* ATCC 35984, *S. epidermidis* HB, *S. epidermidis* CN-899 and *S. haemolyticus* ATCC 43253 were collected, washed and incubated with Mab or PBS alone (control) at a concentration of 2 µg/ml after blocking with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-F<sub>(ab')<sub>2</sub></sub>-Anti-Mouse-F<sub>(ab')<sub>2</sub></sub>-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured. These data indicate that antibodies against *S. aureus* SasA were able to recognize a homologous protein on the surface of coagulase-negative staphylococci. The data support Western blot analysis demonstrating that rabbit polyclonal antibodies against *S. aureus* SasA cross-react with a protein released from the cell surface of *S. epidermidis* HB as well as the recombinant A-region from DgsK cloned from *S. epidermidis* (see Figure 6 and Table 3 below).

**Table 3. Polyclonal Sera Reactivity**

	New man	67-0	397 (SAL 6)	Wo od 46	8325 -4	MRS A 16	ATC C 3598	HB	CN- 899	ATC C 4325
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							4			3
Normal Mouse Sera	-	-	-	-	-	-	-	-	-	-
Mouse anti-SasA	+	+	+/-	-	+	+	+	+	+	+

Although production of antibodies using recombinant forms of the surface proteins of the present invention is preferred, antibodies may be generated from natural isolated and purified versions of these proteins or their active regions such as the A domain, and monoclonal or polyclonal antibodies can be generated using these proteins or active regions in the same manner as described above to obtain such antibodies. Still other conventional ways are available to generate the antibodies of the present invention using recombinant or natural purified proteins or their active regions, as would be recognized by one skilled in the art.

As would be recognized by one skilled in the art, the antibodies of the present invention may also be formed into suitable pharmaceutical compositions for administration to a human or animal patient in order to treat or prevent an infection caused by staphylococcal bacteria. Pharmaceutical compositions containing the antibodies of the present invention, or effective fragments thereof, may be formulated in combination with any suitable pharmaceutical vehicle, excipient or carrier that would commonly be used in this art, including such as saline, dextrose, water, glycerol, ethanol, other therapeutic compounds, and combinations thereof. As one skilled in this art would recognize, the particular vehicle, excipient or carrier used will vary depending on the patient and the patient's condition, and a variety of modes of administration would be suitable for the compositions of the invention, as would be recognized by one of ordinary skill in this art. Suitable methods of administering any pharmaceutical composition disclosed in this application include,

but are not limited to, topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal and intradermal administration.

For topical administration, the composition is formulated in the form of an ointment, cream, gel, lotion, drops (such as eye drops and ear drops), or solution (such as mouthwash). Wound or surgical dressings, sutures and aerosols may be impregnated with the composition. The composition may contain conventional additives, such as preservatives, solvents to promote penetration, and emollients. Topical formulations may also contain conventional carriers such as cream or ointment bases, ethanol, or oleyl alcohol. Additional forms of antibody compositions, and other information concerning compositions, vaccines, methods and applications with regard to other MSCRAMM@s will generally also be applicable to the present invention involving the aforementioned MSCRAMM@s and their active regions and antibodies thereto, and these other MSCRAMM@s are disclosed, for example, in U.S. patents 5,175,096; 5,320,951; 5,416,021; 5,440,014; 5,571,514; 5,652,217; 5,707,702; 5,789,549; 5,840,846; 5,980,908; 6,086,895; 6,008,341; 6,177,084; 5,851,794 and 6,288,214; all of these patents incorporated herein by reference.

The antibody compositions of the present invention may also be administered with a suitable adjuvant in an amount effective to enhance the immunogenic response. For example, suitable adjuvants may include alum (aluminum phosphate or aluminum hydroxide), which is used widely in humans, and other adjuvants such as saponin and its purified component Quil A, Freund's complete adjuvant, RIBBI adjuvant, and other adjuvants used in research and veterinary applications. Still other chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff *et al. J. Immunol.* 147:410-415 (1991) and incorporated by reference herein, encapsulation of the conjugate within a proteoliposome as described by Miller *et al., J. Exp. Med.* 176:1739-1744 (1992) and incorporated by reference herein, and encapsulation of the protein in lipid

vesicles such as Novasome™ lipid vesicles (Micro Vascular Systems, Inc., Nashua, NH) may also be useful.

In any event, the antibody compositions of the present invention which recognize the proteins or their active regions as set forth above will be useful in methods of preventing or treating staphylococcal infection, and in inhibiting binding of staphylococcal bacteria to host tissue and/or cells. In accordance with the present invention, methods are provided for preventing or treating a staphylococcal infection which comprise administering an effective amount of an antibody to the surface proteins as set forth herein or their active subregions so as to treat or prevent a staphylococcal infection. In addition, these monoclonal antibodies will be useful in impairing the binding of staphylococcal bacteria to host cells

Accordingly, in accordance with the invention, administration of the antibodies of the present invention in any of the conventional ways described above (e.g., topical, parenteral, intramuscular, etc.), and will thus provide an extremely useful method of treating or preventing staphylococcal infections in human or animal patients when an effective amount of the antibody compositions are administered to a human or animal patient. By effective amount is meant that level of use, such as of an antibody titer, that will be sufficient to either prevent adherence of the bacteria, to inhibit binding of staph bacteria to host cells and thus be useful in the treatment or prevention of a staph infection. As would be recognized by one of ordinary skill in this art, the level of antibody titer needed to be effective in treating or preventing staphylococcal infection will vary depending on the nature and condition of the patient, and/or the severity of the pre-existing staphylococcal infection.

In addition to use in methods or treating or preventing a staphylococcal infection, the antibodies of the invention may also be used for the specific detection of staphylococcal proteins, or as research tools. The term "antibodies" as used herein includes monoclonal, polyclonal, chimeric, single chain, bispecific, simianized, and humanized or primatized antibodies as well as Fab fragments, such as those fragments which maintain the binding specificity of the antibodies to the



surface proteins specified above, including the products of an Fab immunoglobulin expression library. Accordingly, the invention contemplates the use of single chains such as the variable heavy and light chains of the antibodies. Generation of any of these types of antibodies or antibody fragments is well known to those skilled in the art. In the present case, antibodies to the surface proteins or their active regions as referred to above can be generated, isolated and/or purified, and then used to treat or protect against staphylococcal infection.

Any of the above described antibodies may be labeled directly with a detectable label for identification and quantification of staph bacteria. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances, including colored particles such as colloidal gold or latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA).

Alternatively, the antibody may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin. The antibody may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

In accordance with the present invention, there are also provided vaccines for either active or passive immunization designed to treat or protect against staphylococcal infections, and these vaccines may be prepared from the surface proteins or their active regions as set forth above using a number of the conventional vaccine preparation methods well known in this field. In the typical vaccine, an immunogenic amount of a suitable surface protein or active fragment thereof is combined with a suitable pharmaceutically acceptable vehicle, carrier or excipient, and an amount of this vaccine effective to immunize a human or animal

patient may be administered as appropriate. By immunogenic amount it would be understood by one of ordinary skill in this art that this refers to any amount of the protein or active fragment or subregion thereof which is able to raise an immunogenic response in the human or animal patient.

5 In addition to active vaccines wherein antibodies are generated in the patient by virtue of the introduction or administration of an immunogenic amount of a protein or active fragment in accordance with the present invention, the isolated antibodies of the present invention, or active fragments thereof, may also be utilized in the development of vaccines for passive immunization against staph infections. In  
10 such a case, the antibody compositions as described above, namely an effective amount of the antibody and a pharmaceutically acceptable vehicle, carrier or excipient, may be administered as appropriate to a human or animal patient.

Accordingly, in accordance with the invention, the proteins or active fragments thereof may be utilized as active vaccines, and the antibodies of the  
15 invention may be used as a passive vaccine which will be useful in providing suitable antibodies to treat or prevent a staphylococcal infection. As would be recognized by one skilled in this art, a vaccine may be packaged for administration in a number of suitable ways, such as by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or nasopharyngeal (i.e., intranasal) administration.

20 One such mode is where the vaccine is injected intramuscularly, e.g., into the deltoid muscle, however, the particular mode of administration will depend on the nature of the bacterial infection to be dealt with and the condition of the patient. The vaccine is preferably combined with a pharmaceutically acceptable vehicle, carrier or excipient to facilitate administration, and the carrier is usually water or a  
25 buffered saline, with or without a preservative. The vaccine may be lyophilized for resuspension at the time of administration or in solution.

In addition, in certain cases, the antibodies of the present invention may be modified as necessary so that, when necessary, they become less immunogenic in the patient to whom it is administered. For example, if the patient is a human, the  
30 antibody may be "humanized" by transplanting the complementarity determining

regions of the hybridoma-derived antibody into a human monoclonal antibody as described, e.g., by Jones *et al.*, *Nature* 321:522-525 (1986) or Tempest *et al. Biotechnology* 9:266-273 (1991) or "veneered" by changing the surface exposed murine framework residues in the immunoglobulin variable regions to mimic a homologous human framework counterpart as described, e.g., by Padlan, *Molecular Imm.* 28:489-498 (1991), these references incorporated herein by reference. Even further, when so desired, the monoclonal antibodies of the present invention may be administered in conjunction with a suitable antibiotic to further enhance the ability of the present compositions to fight bacterial infections when necessary.

In addition to treating human or animal patients, the present compositions may also be used to halt or prevent infection of a medical device or other biomaterials such as an implant. Medical devices or polymeric biomaterials to be coated with the antibodies, proteins and active fragments described herein include, but are not limited to, staples, sutures, replacement heart valves, cardiac assist devices, hard and soft contact lenses, intraocular lens implants (anterior chamber or posterior chamber), other implants such as corneal inlays, kerato-prostheses, vascular stents, epikeratophalia devices, glaucoma shunts, retinal staples, scleral buckles, dental prostheses, thyroplastic devices, laryngoplastic devices, vascular grafts, soft and hard tissue prostheses including, but not limited to, pumps, electrical devices including stimulators and recorders, auditory prostheses, pacemakers, artificial larynx, dental implants, mammary implants, penile implants, cranio/facial tendons, artificial joints, tendons, ligaments, menisci, and disks, artificial bones, artificial organs including artificial pancreas, artificial hearts, artificial limbs, and heart valves; stents, wires, guide wires, intravenous and central venous catheters, laser and balloon angioplasty devices, vascular and heart devices (tubes, catheters, balloons), ventricular assists, blood dialysis components, blood oxygenators, urethral/ureteral/urinary devices (Foley catheters, stents, tubes and balloons), airway catheters (endotracheal and tracheostomy tubes and cuffs), enteral feeding tubes (including nasogastric, intragastric and jejunal tubes), wound drainage tubes, tubes used to drain the body cavities such as the pleural, peritoneal, cranial, and

pericardial cavities, blood bags, test tubes, blood collection tubes, vacutainers, syringes, needles, pipettes, pipette tips, and blood tubing.

It will be understood by those skilled in the art that the term "coated" or "coating", as used herein, means to apply the antibody or active fragment, or pharmaceutical composition derived therefrom, to a surface of the device, preferably an outer surface that would be exposed to streptococcal bacterial infection. The surface of the device need not be entirely covered by the protein, antibody or active fragment.

The preferred dose for administration of an antibody composition in accordance with the present invention is that amount will be effective in preventing of treating a staphylococcal infection, and one would readily recognize that this amount will vary greatly depending on the nature of the infection and the condition of a patient. As indicated above, an "effective amount" of antibody or pharmaceutical agent to be used in accordance with the invention is intended to mean a nontoxic but sufficient amount of the agent, such that the desired prophylactic or therapeutic effect is produced. As will be pointed out below, the exact amount of the antibody or a particular agent that is required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition being treated, the particular carrier or adjuvant being used and its mode of administration, and the like. Accordingly, the "effective amount" of any particular antibody composition will vary based on the particular circumstances, and an appropriate effective amount may be determined in each case of application by one of ordinary skill in the art using only routine experimentation. The dose should be adjusted to suit the individual to whom the composition is administered and will vary with age, weight and metabolism of the individual. The compositions may also contain stabilizers or pharmaceutically acceptable preservatives, such as thimerosal (ethyl(2-mercaptobenzoate-S)mercury sodium salt) (Sigma Chemical Company, St. Louis, MO).

When used with suitable labels or other appropriate detectable biomolecule or chemicals, the monoclonal antibodies described herein are useful for purposes

such as *in vivo* and *in vitro* diagnosis of staphylococcal infections or detection of staphylococcal bacteria. Laboratory research may also be facilitated through use of such antibodies. Various types of labels and methods of conjugating the labels to the antibodies of the invention are well known to those skilled in the art, such as the ones set forth below.

For example, the antibody can be conjugated (directly or via chelation) to a radiolabel such as, but not restricted to,  $^{32}\text{P}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{125}\text{I}$ , or  $^{131}\text{I}$ . Detection of a label can be by methods such as scintillation counting, gamma ray spectrometry or autoradiography. Bioluminescent labels, such as derivatives of firefly luciferin, are also useful. The bioluminescent substance is covalently bound to the protein by conventional methods, and the labeled protein is detected when an enzyme, such as luciferase, catalyzes a reaction with ATP causing the bioluminescent molecule to emit photons of light. Fluorogens may also be used to label proteins. Examples of fluorogens include fluorescein and derivatives, phycoerythrin, allo-phycoyanin, phycocyanin, rhodamine, and Texas Red. The fluorogens are generally detected by a fluorescence detector.

The location of a ligand in cells can be determined by labeling an antibody as described above and detecting the label in accordance with methods well known to one skilled in the art, such as immunofluorescence microscopy using procedures such as those described by Warren et al. (*Mol. Cell. Biol.*, 7: 1326-1337, 1987).

As indicated above, the monoclonal antibodies of the present invention, or active portions or fragments thereof, are particularly useful for interfering with the initial physical interaction between a staphylococcal pathogen responsible for infection and a mammalian host, and this interference with the physical interaction may be useful both in treating patients and in preventing or reducing bacteria infection on in-dwelling medical devices to make them safer for use.

In another embodiment of the present invention, a kit which may be useful in isolating and identifying staphylococcal bacteria and infection is provided which comprises the antibodies of the present invention in a suitable form, such as lyophilized in a single vessel which then becomes active by addition of an aqueous

sample suspected of containing the staphylococcal bacteria. Such a kit will typically include a suitable container for housing the antibodies in a suitable form along with a suitable immunodetection reagent which will allow identification of complexes binding to the surface proteins or the antibodies of the invention. In general, these kits may contain an antibody in accordance with the invention and means to identify binding of that antibody when a sample from a patient is introduced to the antibody. For example, a suitable immunodetection reagent may comprise an appropriate detectable signal or label, such as a biotin or enzyme that produces a detectable color, etc., which may be linked to the antibody or utilized in other suitable ways so as to provide a detectable result when the antibody binds to the antigen.

In short, the antibodies of the present invention which recognize and bind to the surface proteins of the invention, or active fragments thereof, will thus be useful in treating a wide variety of staphylococcal infections in human and animal patients and in medical or other in-dwelling devices. In accordance with the invention, because of the nature of these proteins and the fact that they contain epitopes in common with proteins of the other type of staphylococcal bacteria, i.e., a protein from a coagulase-negative staph will raise antibodies that recognize a homologous protein from *S. aureus* and vice versa, the antibodies of the invention will exhibit cross-reactivity and should be effective against a broad range of staphylococcal infections. Accordingly, the present invention provides methods and compositions for improved methods of treating or protecting against a wide range of staphylococcal infections.

## EXAMPLES

The following examples are provided which exemplify aspects of the preferred embodiments of the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure,

appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

#### 5 **Example 1. Isolation and Sequencing of MSCRAMM's from *S. Aureus***

*Staphylococcus aureus* is known to express a class of surface-associated proteins which play important roles in pathogenicity by allowing bacteria to avoid host defenses and by acting as adhesins. These proteins are known as MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules) and in  
 10 most cases are covalently anchored to the cell wall peptidoglycan. They have several common features: (i) an N-terminal signal peptide (approximately 40 residues in length) required for Sec-dependent secretion, (ii) a wall spanning domain either rich in proline and glycine residues or composed of serine and aspartate dipeptide repeats, (iii) an LPXTG motif required for covalent anchoring of  
 15 the protein to the pentaglycine crossbridge in peptidoglycan, (iv) a hydrophobic membrane-spanning domain followed by (v) several positively charged residues.

By exploiting the whole genome sequences of *S. aureus*, eight novel open reading frames encoding proteins with secretion and anchorage motifs indicative of MSCRAMMs were identified (i.e. bearing an N-terminal signal peptide and a C-  
 20 terminal LPXTG motif followed by a hydrophobic domain and a positively charged tail). The following Table illustrates the list of proteins identified including their distribution among *S. aureus* genomes, their protein size and C-terminal cell wall sorting sequence.

Name	Distribution	Size	C-terminus
EkeS	ENCSJM	2189 aa	LPNTGSEEMDLPLKELALITGAALLARRRS KKEKES
DsqA	ENCSJM	~1363- 2283 aa	LPDTGDSIKQNGLLGVM TLLVGLGLMKR KKKKDENDQDDSQA
KesK	ENCSJM	~909 aa	LPKTGETTSSQSWWGLYALLGMLALFIPK FRKESK

<b>KrkN2</b>	ENCSJM (Cowan)	~278 aa	<b>LPKTGLTSVDNFISTVAFATLALLGSLSLLLF KRKESK</b>
<b>KrkN</b>	ENCSJM	~661 aa	<b>LPQTGEESNKDMTLPMLIALSSIVAFVLP RKRRKN</b>
<b>RkaS</b>	ENCSJM	~801 aa	<b>LPKTGTNQSSSPEAMFVLLAGIGLIATVRR RKAS</b>
<b>RrkN</b>	NCSJM	1629 aa	<b>LPKTGLESTQKGLIFSSIIGIAGLMLLARRRK N</b>
<b>KnkA</b>	NCSJM	629 aa	<b>LPKAGETIKEHWLPISVIVGAMGVLMIWLS RRNKLKNKA</b>

Abbreviations: eMRSA-16; N, 8325; C, COL; S, MSSA; J, N315, M, Mu50.

Six out of eight are conserved in all of the six staphylococcal genomes currently sequenced and the remaining two are present in 5/6 of these genomes.

5

The following is a list of the DNA and protein sequences:

Ekes MRSA (SEQ ID NO:1)

10 acaacacagcagagaatagacaaccaggaggaaaacgaaatgaatttgtaaagaaaaataaatatagtattag  
aaaatataaagtaggatattctactttaatcgggacagtttattactttcaaaccctaatggtgcacaagctttaac  
tacggatcataatgtgcaaggtggttcaaatcaagcattacctggcaactcacaatacaaatgccgataactc  
gagacatagtaaagtattcgcaaaatactcctaattgcacatgcaacagacaatacatcaacaaatcaagcattgac  
15 taatcatcaaaacgttgatgtggcaaatcaagtcgggcctgtcctaatacagcctagcgcgtcgccgcaaaata  
ataataattctaattgctaattcaacagcaacagagccagcggcgaatacaataataatttagcatcaataacaat  
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g t t a a t a g a a a a g t t g a t g a c a t g g a a g a t t t a g t t a a c c a a a a t g a t g a a c t g a c a g a t g a a g a a a a c a a g c a  
g c g a t t c a a g t c a t c g a g g a a c a t a a a a a t g a a t t a t t g g g a a t t a t t g g t g a c c a a a c g a c t g a t g a t g g c g t t a c t  
a g a a t t a a g a t c a a g g t a t a c a g a c t t a a g t g g a g a c a c t g c a a c a c c a g t t g t t a a c c a a a t g c t a a a c a g  
c t a t a c g t g a t a a a g c a g c g a a a c a a g a g a a a t t a c a a t c a c a c g c c a g a t g c t a c t c a a g a t g a a a t t c a a g  
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a a c a g c t a a a a a t a a t g g t a t t a a c a a t t g g t g c a g t t g c c c a a a g t g a c a c a a a c a a g c t g c a a g a g a  
t g c a a t t a a t c a a g c g a c a g c a a c g a a a c g a c a a a a t a a t a g c a a t a g a g a g c a a c a c a a g a g a g a  
a a a a t g c a g c a t t g a a t g a a t t a a c g c a a g c c a c g a a c c a c g c a t t a g a a c a a a t c a a t c a a g c g a c a a c c a a t  
g a t g a t g t a g a t a c t g c c a a a g g t g a t g g t c t g a a t g c c a t t a a t c c t a t t g c g c c t g t a a c t g t t g c a a g c a a g c a g  
10 c a a g a g a t g c c g t a t c a c a t g a t g c a c a a c a g c a t a t c g c a g a g a t c a a t g c a a a t c c t g a t g c g a c t c a a g a a g  
a a a g c a a a g c a g c a a t a g a g a a g t a a a t g c t g t g a t g t g t g c g a a t a c t a a t a t a t t a a a t g c t a a t a c c a a t  
g c t g a t g t t g a c a a g t a a a g a c a a a t g c a a t t c a a g g t a t a c a a g c c a t t g a a c c a g c t a c a a g g t t a a a c a  
g a t g c t a a a a c g c t a t t g a t c a a a g t g c g g a a a c g c a a c a t a a t g c g a t a t t a a t a a t a a t g a t g c g a c c t t a g a  
a g a g c a a c a a g c a g c a c a c a a a t t g c t g a t c a a g c t g a c c a c a g c g a a g c a a a a t a t t a a t g c a g c a g a t a  
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a a a c g g a t g c a c g c a a t g c t g t a a a t g a a a a a g c g c g a g a g g c g a t a a c a a a t a t c a a t g c t a c a c c t g g c g c g  
a c t c g a g a a g a g a a a c a a g a a g c g a t a a a t c g t g c a a t a c a c t t a a a a t a g a g c a t t a a a t g a t a t t g g t g t g a  
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a g a a a c a a a c t g c t a c a g g t g t a t t a a c g g a c t a g c a a c t g c a a a a a a c a a g a a a t a a t c a a a a t a c a a a t g  
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a a g c t g a t a c t a a t g c a g a a g t a g a t c a a g c a c a a a t t a g g t a c a a a a g c a a t t a a t g c g a t t c a g c c a a a t a t  
t g t a a a a a a a c c t g c a g c a t t a g c a c a a c c a a t c a g c a t t a t a g t g c t a a a t t a g t t g a a a t c a a t g c t a c a c c a g  
a t g c a a c a g a t g a t g a g a a a a t g c t g c g a t c a a t a c t t a a a t c a a g a c a g a c a a c a a g c t a t t g a a a g t a t t a a  
a c a a g c a a a t a c a a a t g c g g a a g t a g a c c a a g c t g c g a c a g t g g c a g a g a a t a t c g a t g c t g t t c a a g t t g a  
25 c g t t g t a a a a a a c a a g c a g c g c g a g a t a a a t c a c t g c t g a a g t a g c g a a g c g t a t t g a a g c g g t t a a c a a a  
c a c c t a a t g c a a c t g a c g a a g a a a a g c a g g c t g c a g t t a a t c a a t c a a t c a a c t t a a a g a t c a a g c g t t a a t c a  
a a t t a a t c a a a c c a a a c a a a t g a t c a g g t a g a c g c a a c t a c a a a t c a a g c g a t t a a t g c t a t a g a t a a t g t t g a a  
g c t g a a g t a g t a a t t a a c c a a a g g c a a t t g c a g a t a t t g a a a a g c t g t t a a a g a a a a g c a a c a g c a a a t t g a t  
a a t a g t c t t g a t t c a a c a g a t a a t g a g a a g a a g t t g c t t a c a a g c a t t a g c t a a a g a a a a g a a a a g c a c t t g  
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c a a c c t g a a a c a a a a a t t a a a c c a g c a g c a c g t g a a a a a t c a a t c a a a a a g c g a a t g a a t t a c t g c g c a a a  
t t a a t c a a g a t a a a g a a g c g a c a g c a g a a g a a g a c a a g c g g c g t g a t a a a a t c a a t g a t t a g t t g c t a a a g  
c t a t g a c a a a t a t c a c g a a t g a t a g a c a a a t c a g c a a g t t a a t g a c t a c a a a a t c a a g c g c t t g a c g a c a t t g c  
a t t a g t g a c g c c t g a c c a t a t t g t t a g a g c a g c t g c t a g a g a t g c a g t t a a g c a c a a t a t g a a g c t a a a a g c a c  
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c g t g c a t t a c a a a c a t t a a t c a a g c a a t a g c g a a t a t g a t g t g a a a c g t g t t a a t c a a a t g g t a t t g c t a c g t t a a  
a a g g c g t a g a a c c g c a c a t t g t g g t t a a c c t g a a g c t c a a g a a g c c a t a a a a g c g a g c g a g a t a a c c a a g t a  
g a a t c t a a a a g a t a c a c c a c a t g c t a c g a c a g a t g a a t t a g a t g a a g c a a c c a c a a a t a a a c g a c a c a c t t  
a a c a a g g t c a a c a a g a t a t a g a c a a t a c g a c a c a a g a t g c a g c t g c a a t g a t g t t a g a a c c a a a c g a t t a a  
40 g g c a a t c g a a c a a a t t a a a c c g a a a g t t a g a c g c a a a c g t g c a g c g t t g g a t a a c a t t g a t g a a g t a a t a a t a a t  
c a a c t c g a t g c a a t a c g a a a t a c g c t a g a t a c a a c g c a a g a t g a a c g a a a t g t t g c t a t t g c t g c g t t a a a t a a a t  
t g t t a a t g c a a t t a a a a t g a t a t t g c a c a a a c a a a a c g a a t g c a g a a g t g g a t c a a a c t g a g g c t g a t g g t a a c  
a a c a a t c a a a g t g a t t t a c c t a a a g t t c a a g t t a a a c c a g c a g c g c t c a a t c t g t c a g c g a a a a g c t g a a g  
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45 c a c t t a a t c a a g c t a t t g a t c a g a t c a a t c a c g c a g a t a a g a c t g c g c a a g t t a a t c a a a a t a g t a t c g a t g c t c a a a  
a t a t t a t t c a a a a t t a a a c c a g c g a c a a c a g t t a a g c a a c a g c a t t a c a c a a a t t c a a a a t a t c g c t a c a a t

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ataa

EkeS\_MRSA (SEQ ID NO:2)

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DsqA (8325) (SEQ ID NO:3)

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DsqA (8325) (SEQ ID NO:4)

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20 HGNGGDGIGFAFSPGVLGETGLNGAAVGIGGLSNAFGFKLDYHNTSKPNSA  
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25 DNGTGT VTNVTGLPSGLSYDSATNSIIGTPKIGQSTVTVVSTDQANNKSTTTFTI  
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35 ESDSISESTSTSDSISEAISASESTFISLSESNSTSDSESQSASAFLESESTSES  
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STSNRMSTIASLSTSVSTSESGSTSESTSESDSTSTSLSDSQSTSRSTASGSAST  
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45 SDSKSMGSGSTSVSDGSLSVSTSLRKSESVSESSSLSCSQMSMSVSTSDSSSL  
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5 SDSTSTSTSDSTSGSTSTSISESLSTSGSGSTSVSDSTSMSESNSSSVSMSQDKS  
DSTSIDSESVSTSTSTSLSTSDSTSTSESLSTSMGSQSISDSTSTSMGSTST  
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## 10 KesK1 (8325) (SEQ ID NO:5)

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KesK1 (8325) (SEQ ID NO:6)

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NKEHDIGPREQVNFQLLDKNNETQYYHFFSIKDPADVYYTKKKAVELDINTASTW  
10 KKFVYENNQKLPVRLVSYSPVPEDHAYIRFPVSDGTQELKIVSSTQIDDGEETNY  
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VKIVSSIEYGENIHEDYDYLTMVFAQPITNNPDDYVDEETYNLQKLLAPYHKAKTLE  
15 RQVYELEKLQEKLPEKYKAEYKKKLDQTRVELADQVKSAVTEFENVTPNTDQLTD  
LQEAHFVVFEESEENSESVMDFVEHPFYATLNGQKYVVMKTKDDSYWKDLIVEG  
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20 GMSSNVDTDKDSNKNKDKVIQLNHIADKNNHTGKAAKLDVVKQNYNNTDKVTDKK  
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KrkN2 (8325) (SEQ ID NO:7)

25  
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45 KrkN2 (8325) (SEQ ID NO:8)



EENNMTKHYLNSKYQSEQRSSAMKKITMG TASIILGSLVYIGADSQQVNAATEATN  
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QINYNHRYTTHLEFEKAIPTLADAAKPNNVKPVQPKPAQPKTPTEQTKPVQPKVEK  
5 VKPTVTTTTSKVEDNHSTKVSTDTTKDQTKTQTAHTVKTAQTAQE QNKVQTPVKD  
VATAKSESNNQAVSDNKSQQTNKVTKHNETPKQASKAKELPKTGLTSVDNFISTV  
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KrkN (8325) (SEQ ID NO:9)

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35 gcaaaatcattaccacaaactggtgaagaatcaataaagatatgacattaccattaatggcattattagctttaagta  
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KrkN (8325) (SEQ ID NO:10)

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KAPKETKEVKPAAKATNNTYPILNQELREAIKNPAIKDKDHSAPNSRPIDFEMKKKD  
GTQQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEGDKKLPIKLVSYD  
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45 YKAEKLLAPYKAKTLERQVYELNKIQDKLPEKLKAEYKKKLEDTKKALDEQVKS  
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METTNDYWKDFMVEGQVRVTRISKDAKNNTRTIIFPYVEGKTLYDAIVKVHVKTIDY  
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QDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKGEVSSSTPTKVSTTQ  
5 NVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQKANIKNTNDGHTQSQNNKNTQE  
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RkaS (COL) (SEQ ID NO:11)

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RkaS (COL) (SEQ ID NO:12)

45 FINNLHKINHFNIRIMIYWCM TVN GG NEMKALLL KTSVWL VLLFSVMGLWQVSNA  
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QGLPLSNQSKGEEMAKAMNAVGYDAMAVGNHEFDFGYDQLKKLEGMLDFPMLS  
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5 EMMRIYKDVDTFVISHLGIDPSTQETWRGDYLVKQLSQNPQLKKRITVIDGHSHT  
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AEQINQADQTFRAQTAEVII PNNTIDFKGERDDVTRTRETNLGNAIADAMEAYGVKN  
FSKKTDFAVTNGGIRASIAKGKVTRYDLISVLPFGNTIAQIDVKGSDVWTA FEHSL  
GAPTTQKDGKTVLTANGGLLHISDSIRVYYDINKPSGKRINAIQILNKETGKFENIDL  
10 KRVYHVTMNDFTASGGDGYSMFGGPREEGISLDQVLASYLKTANLAKYDTTEPQR  
MLLGKPAVSEQPAKGQQGSKGSKSGKDTQPIGDDKVM DPAKKPAPGKVLLLAH  
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EAMFVLLAGIGLIATVRRRKAS

15

RrK N (8325) (SEQ ID NO:13)

20

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35 aagagattccattcaagaaagaacgtaaatattaatccggtatttagcaccagggaacagaaaaagtaacaagagaa  
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gaacaaaaagaagagattacaaaagatccgattaatgaattaacagaatacggacctgaaacaatagcgccag  
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aatccagaaacaggagacgttagtagaccgctgtagatagcgtaacaaaatatggacctgtaaaaggagactc  
40 gattgtagaaaaagaagagattccattcgagaagaacgtaaatattaatcctgatttagcaccagggaacagaaaaa  
gtaacaagagaaggacaaaaagggtgagaagacaataacgacgccaacactaaaaaatccattaactggagaa  
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45 aaaaggagactcgattgtagaaaaagaagagattccattcgagaaagaacgtaaatattaatcctgatttagacca  
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30 ccaaaaacgggtacaccagaaacaaaaacagtagagataccgtttgaaacaaaacgtgagtttaacaaaatt  
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gaaccattaacagggtgaaaaagttggcgagggtcaaccaacagaagagatcacaaaacaaccagtagataa  
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35 tcattcaatggataaaaatgataaagttaaaaaatctaaaattgctaaagaatcagtagctaatcaagagaaaaaa  
cgagcagaattacaaaaaacagggttagaaagcacgcaaaaagggttgatcttagtagtataattggaattgctgga  
ttaatgttattggctcgtagaagaaagaattaa

RrkN (8325) (SEQ ID NO:14)

40 SGKYGKRSMQMRDKKGPVNKRVDFLSNKLNKYSIRKFTVGTASILIGSLMYLGTQ  
QEAEAAENNIENPTTLKDNVQSKEVKIEEVTNKDTAPQGVEAKSEVTSNKDTIEHE  
PSVKAEDISKKEDTPKEVADVAEVQPKSSVTHNAETPKVRKARSVDEGSFDITRDS  
KNVVESTPITIQQKEHFEGYGSVDIQKKPTDLGVSEVTRFNVGNESNGLIGALQLK  
45 NKIDFSKDFNFKVRVANNHQSNTTGADGWGFLFSKGNAAEYLTNGGILGDKGLVN  
SGGFKIDTGYIYTSSMDKTEKQAGQGYRGYGAFAVKNDSSGNSQMVGENIDKSKT

NFLNYADNSTNTSDGKFHGQRLNDVILTYVASTGKMRAEYAGKTWETSITDLGLS  
KNQAYNFLITSSQRWGLNQGINANGWMRTDLKGSEFTFTPEAPKTITELEKKVEEI  
PFKKERKFNPDLAPGTEKVTREGQKGKEKTITPTLKNPLTGVIISKGEPKEEITKDP  
5 NELTEYGPETIAPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTKY  
GPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGKEKTITPTLKNPLTGEI  
SKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETG  
DVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGKEK  
TITPTLKNPLTGVIISKGEPKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKE  
10 EVPGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFKKERKFNPDLAPG  
TEKVTREGQKGKEKTITPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGH  
RDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIP  
FEKERKFNPDLAPGTEKVTREGQKGKEKTITPTLKNPLTGEIISKGESKEEITKDPIN  
ELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVRPPVDSVTKYG  
PVKGDSIVEKEEIPFKKERKFNPDLAPGTEKVTREGQKGKEKTITPTLKNPLTGEIIS  
15 KGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGD  
VVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTREGQKGKEKT  
TPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEE  
VPGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGT  
EKVTREGQKGKEKTITPTLKNPLTGEIISKGESKEEITKDPVNELTEFGGEKIPQGH  
20 KDIFDPNLPTDQTEKVPKPGIKNPDTGKVIIEPVDDVIKHGPKTGT PETKTVEIPF  
ETKREFNPQLQPGEEVKQEGQPGSKTITPITVNPLTGEKVGEGQPTTEEITKQPV  
DKIVEFGGEKPKDPKGPENPEKPSRPTHPSGPVNPNNPGLSKDRAKPNGPVHSM  
DKNDKVKKSKIAKESVANQEKKRAELPKTGLESTQKGLIFSSIIGIAGLMLLARRRK  
N

KnkA (8325) (SEQ ID NO:15)

ggaaggagtagtgtagggctaaatatcgagggaacccgtttcaattatatgtaaagttatcgtgttcgacaatgatggc  
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30 gatattatctaagcaagatttattagacaaagtgacaaggcaattcgtcaaattgagcaattaaaaacagttatcggtt  
catctaaagaacattataaagcacaactaaatgaagcgaacacagcatcgcaaatagatgaaatcataaaacga  
gctaattgagttgtagcaaaagacaataaaagttctcacactgaaatgaacgggtcaaagtatatagacagtaaatt  
agatcaattgctaaagatttaaagaggtttctcaaatgttgataggggtcaacaaagtgaggacgatcttaatt  
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35 atggtaataaaggcgtagaagacctagaccattgaatcagcaatacacaaatcgaaagatgcacgaaagat  
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40 gaagtaaataagacgaaagagcgataaaaagtcacgaaatatttttgaagaactgcacgtactgatgata  
aaaagtatgtacacaaagcatttagaaagtataTTtaaaagacgaggcagttaaaattctaaaagataacgt  
gttgatggtaaaacagatcaacaaatgcagatcaaatfactcgtcatattgatcaattatctctgacaacgagtgatg  
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ctgaagcagataaaattggctaaagattggacgaataaaggattatcaaatcgccaaatcggtgaccaattgaagaa  
45 acattttgatcaactggcgacacgtctcagatgatataTTaaaagcaattttgaataatgccaaagataaaaaaca  
agcaattgaaacgatttagcaacacgtatagaaagacaaaaggcaaaattactggcagatttaattactaaaata

gaaacagatcaaaataaaattttaatttagttaaatcggcattgaatggtaaagcggatgattattgaattacaaaa  
 gagactcaatcaaacgaaaaagatatagattatatttatcaccaatagtaaatcgccaagttactagatcgattg  
 aataaaaaatgggaaaacgacagatttaataagttagcaaattaatgaatcaaggatcagattatttagacagtatt  
 ccagatataccacaccaaagccagaaaagacgttaacacttggttaaaggtaatggattgtaagtggattattaaa  
 5 tgctgatggtaatgtatctttgcctaaagcgggggaaacgataaaagaacattggttgccgatatctgtaattgttggtg  
 caatgggtgtactaatgatttggtatcacgacgcaataagttgaaaaataaagcataa

KnkA (8325) (SEQ ID NO:16)

GRSMLMAKYRGKPFQLYVKLSCSTMATSILTNILPYDAQAAASEKDTEITKEILSK  
 QDLLDKVDKAIRQIEQLKQLSASSKEHYKAQLNEAKTASQIDEIHKRANELDSKDNK  
 SSHEMNGQSDIDSKLDQLLKDLNEVSSNVDRGQQSGEDDLNAMKNDMSQTATT  
 KHGEKDDKNDEAMVNKALEDLDHLNQQIHKSKDASKDTSEDPAVSTTDNNHEVA  
 KTPNNDGSGHVVLNKLFLSNEENQSHSNRLTDKLQGS DKINHAMIEKLAKSNASTQ  
 15 HYTYHKLNTLQSLDQRIANTQLPKNQKSDLMSEVNKTKERIKSQRNIILEELARTDD  
 KKYATQSILESIFNKDEAVKILKDIRVDGKTDQQIADQITRHIDQLSLTTSDDLTLTSLID  
 QSQDKSLLISQILQTKLGKAEADKLAKDWTNKGLSNRQIVDQLKKHFASTGDTSSD  
 DILKAILNNAKDKKQAIETILATRIERQKAKLLADLITKIETDQNKIFNLVKSALNGKAD  
 DLLNLQKRLNQTKKDIDYILSPIVNRPSLLDRLNKNKGTDDLNLKLANLMNQGSDDL  
 20 SIPDIPTPKPEKTLTLGKGNGLLSGLLNADGNVSLPKAGETIKEHWLPISVIVGAMG  
 VLMIWLSRRNKLKNKA

#### Primary structure analysis:

A bioinformatic approach was used for primary structure and function prediction (Figure 1). Proteins RrkN and DsqA possessed a similar structural organization to previously described MSCRAMMs. RrkN is similar in structure to the Pls/Aap proteins of *S. aureus* and *S. epidermidis*, respectively. It contains a 200-residue domain at its N-terminus showing 40% identity to Pls and Aap. The C-terminus of the protein is predominantly composed of a 128 residue repeat domain, which varies in the numbers of repeats from strain to strain. These repeats are also present in Pls and Aap. A putative *sar* homolog and *fnbpA* and *fnbpB* lie directly upstream from RrkN on the genome.

DsqA is similar in structural organization to the Sdr family of proteins. It contains a typical A domain followed by a TYYFTDVK motif which is similar to a conserved TYTFTVYVD motif found in all of the Sdr proteins. The function of this motif has yet to be determined. Two 88 residue repeat domains reside in the centre of the protein

followed by a C-terminal SX-repeat motif similar to the SD-repeat motif found in the Sdr proteins. The size of this repeat varies from strain to strain. DsqA neighbors *secY* and *secA* on the genome. A DsqA homolog (>90% identical) is also found in *S. epidermidis*.

5

KnkA contains no repeat domains in its sequence. Secondary structure prediction analysis indicate that this protein is predominantly composed of alpha-helices.

RkaS contains no repeat domains in its sequence. BLAST analysis indicates that it is similar to a 5' nucleotidase UDP-sugar hydrolase. The gene encoding RkaS lies directly upstream from *orfX*, the insertion site of the *mec* element.

10

KesK contains two 140 residue repeat domains at the N-terminus of the protein which are 38% identical. Hydropathy plot analysis (Kyte and Doolittle, 1982) indicates that there is a large hydrophilic domain in the center of the protein (residue 500-560).

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EkeS contains two 300 residue repeat domains in the center of the protein which are 38% identical. Blast analysis indicates that the N-terminus of the protein (residues 1-1268, bearing both repeats) is 49% identical to FmtB, an LPXTG protein with 17 tandem repeats. FmtB is proposed to be involved indirectly in methicillin resistance as inactivation of *fmtB* abolishes methicillin resistance. This appears to be due to affecting cell wall composition as methicillin sensitivity can be relieved by increasing the production of the cell wall precursor glucosamine-1-phosphate (Komatsuzawa *et al.*, 2000).

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KrkN and KrkN2 neighbor each other on the genome.

#### Expression analysis:

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Due to lack of sequence homology with protein databases, a putative function for each of these proteins could not be predicted and hence a molecular approach was taken. Unique regions of four of the *orfs* were expressed in *E. coli* as recombinant his-tagged fusion proteins using the Qiagen pQE-30 expression system. Figure 2. represents a Coomassie stained SDS-PAGE gel of the purified N-terminal his-tag fusion proteins. The recombinant proteins RrkN1, DsqA2, KesK1 and KnkA were used to generate antibodies in rabbits. Western blotting analysis of *S. aureus* cell wall extracts revealed that KesK, KnkA and DsqA are expressed and cell wall-associated (Figure 3). Strain eMRSA-16 represents a *knkA*-negative strain since it lacks the *knkA* gene. An immunoreactive band of 65kDa reacts with the cell wall fraction from both exponential and stationary phase cells of strain 8325-4 (Figure 3, B). The absence of this band in strain eMRSA-16 suggests that it represents the gene product of *knkA*.

Western immunoblotting of the cell wall fraction of strain 8325-4 using anti-KesK antibodies identified a 150kDa immunoreactive band in both exponential and stationary phase cultures. A similar sized immunoreactive protein released from the cell wall fraction of *Lactococcus lactis* expressing full length KesK on an expression plasmid (pKS80) suggests that the 150kDa band represents the *kesK* gene product (data not shown). A *kesK* knockout mutant in *S. aureus* would be required to confirm the size of the cell wall-released KesK protein.

Western immunoblotting of the cell wall fraction of *S. aureus* strain MSSA and eMRSA-16 using anti-DsqA antibodies identified a 130kDa immunoreactive band. Expression levels are higher in stationary phase cells.

#### **Heterologous expression in *Lactococcus lactis*:**

Heterologous expression of *S. aureus* surface proteins in *Lactococcus lactis* (*L. lactis*) has previously been used as a tool to study protein function (Sinha *et al.*, 2000). In this study this surrogate system will be used to express each of the in

silico-predicted MSCRAMMs on the surface of *L. lactis* to fish for a function. KesK and KnkA have been cloned into *L. lactis* and shown by dot blotting to be surface expressed (Figure 4). No cross reaction was observed with the negative control (pKS80 plasmid without an insert) indicating that this is a specific reaction. Cell wall and protoplast fractions of *Lactococcus lactis* bearing pKS-KnkA and pKS-KesK were generated by digestion of cells with lysozyme and mutanolysin and used in Western blotting studies using anti-KnkA and anti-KesK antibodies, respectively. Unlike what was observed in *S. aureus*, KnkA was not detected in the cell wall fraction of *L. lactis* but found to be associated with the protoplast fraction. The anchoring motif of KnkA differs from the consensus LPXTG sequence in that it contains an Alanine residue instead of a Threonine (i.e. LPKAG) (Table 1). It has been recently been published that *S. aureus* contains two sortase genes, *srtA* and *srtB* (Pallen, 2001). It is possible that this variant form of the LPXTG motif is processed by the second sortase gene, which is absent in *L. lactis*. This would also explain the slight increase in size of the KnkA protein observed in the protoplast fraction, as the cell wall sorting signal has not been cleaved.

KesK was detected in the cell wall fraction of *L. lactis* but migrated at a smaller molecular weight than the KesK protein released from the cell wall of *S. aureus*. The majority of MSCRAMMs expressed on the surface of *L. lactis* are prone to proteolysis during the cell wall extraction procedure (Louise O'Brien, personal communication). Therefore, it is possible that the KesK protein released from the surface of *L. lactis* represents a truncated form of KesK. Shorter digestion times with lysozyme and mutanolysin has been shown to limit the extent of proteolysis.

#### **Expression of in silico-predicted MSCRAMMs in vivo:**

Convalescent-phase sera from 33 patients recovering from *S. aureus* infections were tested in their ability to recognize the purified N-terminal his-tag fusion proteins in an ELISA assay. Pooled sera from children and healthy blood donors were used



as negative controls. A positive reaction was taken as a value equal to or greater than twice the value of the negative control. Figures 5A-5D illustrate that all of the proteins were recognized by 27-42% of the patients suggesting that these proteins are expressed *in vivo* and are immunogenic during infection of the host.

5

#### References:

- 10 Komatsuzawa, H., Ohta, K., Sugai, M., Fujiwara, T., Glanzmann, P., Berger-Bachi, B., Suginaka, H. (2000) Tn551-mediated insertional inactivation of the *fntB* gene encoding a cell wall-associated protein abolishes methicillin resistance in *Staphylococcus aureus*. J. Antimicrob. Chemother. **45**: 421-31.
- 15 Sinha, B., Francois, P., Que, Y.A., Hussain, M., Heilmann, C., Moreillon, P., Lew, D., Krause, K.H., Peters, G., Herrmann, M. (2000) Heterologously expressed *Staphylococcus aureus* fibronectin-binding proteins are sufficient for invasion of host cells.  
Infect. Immun. **68**: 6871-6878.
- 20 Pallen, M.J., Lam, A.C., Antonio, M., Dunbar, K. (2000) An embarrassment of sortases - a richness of substrates? Trends. Microbiol. **9**: 97-101

#### Example 2. Isolation and Sequencing of Cross-Reactive Proteins from *S. Aureus* and from Coagulase-Negative Staphylococci

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It has been recently shown that *S. epidermidis* contains surface proteins structurally related to *S. aureus* MSCRAMM<sup>®</sup> proteins (US 09/386,962). One protein from *S. aureus* is of particular interest since it has a close homologue in *S. epidermidis*. The protein is called DsqA or SasA (*S. aureus*) and DgsK (*S. epidermidis*). They are

30 characterized by a typical "A" domain of approximately 500 amino acid residues,

followed by two B repeats of 88 residues that are ~40% identical, and a unique SXXS dipeptide repeat that can vary in length depending on the strain. Contained within the A domain of the *S. aureus* DsqA/SasA is a 180 residue region that has ~40% identity to a similar sized domain within region A of *S. aureus* proteins RrkN, Pls and *S. epidermidis* protein Aap. The A regions of the DsqA/SasA and DgsK proteins are 46 % identical at the amino acid level, the BB repeats are 50% identical. Active and passive immunization strategies that include; vaccines, polyclonal and monoclonal antibodies recognizing both *S. aureus* and coagulase-negative staphylococcal proteins are the subject of this invention.

### Specific Examples of Antibodies that Cross-React with Coagulase-Negative Staphylococci and *S. aureus*.

#### Coagulase-negative staphylococcal DgsK A-Domain:

Amino Acid Sequence (SEQ ID NO:17)

ASETPITSEISSNSETVANQNSTTIKNSQKETVNSTSLASNHSNSTNKMSSSEVTN  
TAQSSEKAGISQQSSETSNQSSKLNTYASTDHVESTTINNDNTAQDQNKSSNVT  
SKSTQSNSTSSSEKNISSNLTQSIETKATDSLATSEARTSTNQISNLTSTSTSNQSSP  
TSFANLRTFSRFTVLNTMAAPTTTTSTTTSSLTSNSVNVNKNDFNEHNMNLGSGSATY  
DPKTGIATLTPDAYSQKGAISLNRDLSNRSFRFIGKVN LGNRYEGYSPDGVAGGD  
GIGFAFSPGPLGQIGKEGA AVGIGGLNNAFGFKLD TYHNTSTPRSDAKAKADPRN  
VGGGGAFGAFVSTD RNMATTEESTA AKLNVQPTD NSFQDFVIDYNGDTKVM TV  
TYAGQTFTRNLTDWIKNSGGTTFSLSMTASTGGAKNLQQVQFGTFEYTESAVAKV  
RYVDANTGKDIIPPKTIAGEVDGTVNIDKQLNNFKNLGYSYVGT DALKAPNYTETSG  
TPTLKL TNSSQTVIYKFKDVQ

#### *S. aureus* SasA A-domain:

Amino Acid Sequence (SEQ ID NO:18)

ASDAPLTSELNTQSETVGNQNSTTIEASTSTADSTSVTKNSSSVQTSNSD TVSSEK  
SEKVTSTTNSTSNQQEKLSTSESTSSKNTTSSSDTKSVASTSSTEQPINTSTNQS  
TASNNTSQSTTPSSVNLNKTSTTSTSTAPVKLR TFSRLAMSTFASAATTTAVTANTI  
TVNKDNLKQYMTTSGNATYDQSTGIVTLTQDAYSQKGAITLGTRIDSNKSFHFSGK  
VNLGNKYEGHGNGGDGIGFAFSPGVLGETGLNGAAVGIGGLSNAFGFKLD TYHNT  
SKPNSAAKANADPSNVAGGGAFGAFVTTDSYGVATTYTSSTADNAAKLN VQPT  
NNTFQDFDINYN GDTKVM TVKYAGQ TWTRNISDWIAKSGTTNFSLSMTASTGGAT  
NLQQVQFGTFEYTESAVTQVRYVDVTTGKDIIPPKTYSGNVDQVVTIDNQQSALTA  
KGYNYTSVDSSYASTYNDTNKTVKMTNAGQSVTYFTDVV

The entire sequence of the Aap protein and the DNA coding therefor (with an indication of the presence of the A domain) is shown below:

***S. epidermidis* Aap Protein (A-domain underlined) (SEQ ID NO:19)**

5  
MGKRRQGPINKKVDFLPNKLNKYSIRKFTVGTASILLGSTLIFGSSSHEAKAAEEKQ  
VDPITQANQNDSSERSLENTNQPTVNNEAPQMSSTLQAEEGSNAEAPQSEPTKA  
EEGGNAEAAQSEPTKAEEGGNAEAPQSEPTKAEEGGNAEAAQSEPTKTEEGSNV  
KAAQSEPTKAEEGSNAEAPQSEPTKTEEGSNAKAAQSEPTKAEEGGNAEAAQSE  
10 PTKTEEGSNAEAPQSEPTKAEEGGNAEAPQSEPTKTEEGGNAEAPNVPTIKANS  
NDTQTQFSEAPTRNDLARKEDIPAVSKNEELQSSQPNTDSKIEPTTSEPVNLNYSS  
PFMSLLSMPADSSSNNTKNTIDIPPTTVKGRDNYDFYGRVDIESNPTDLNATNLTR  
YNYGQPPGTTTAGAVQFKNQVSFDKDFDNIRVANNRQSNTTGADGWGFMFSK  
KDGDDFLKNGGILREKGTPSAAGFRIDTGYNNNDPLDKIQKQAGQGYRGYGT  
15 FVK  
NDSQGNTSKVSGTGSTDFLNYADNTTNDLDGKFHGQKLNNVNLKYNASNQ  
TFT  
ATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNGNSGTASGVMRADLDGATL  
TYTPKAVDGDPPIISTKEIPFNKKREFDPNLAPGTEKVVQKGEPGIETTTTPT  
TYVNP  
TGEKVGEGEPTTEKITKQPVDEIVHYGGEEIKPGHKDEFDPNAPKGSQTTQ  
PGKPG  
VKNPDTGEVVTTPVDDVT  
20 KYGPVDGDPITSTEEIPFDKKREFNPDLKPGEERV  
KQ  
KGEPGKTITTTPTTKNPLTGEKVGEGEPTTEKITKQPVDEITEYGGEIKPGH  
KDEFD  
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KYGPVDGDPITSTEEIPFDKK  
REFNPDLKPGEERVKQKGEPGKTITTTPTTKNPLTGEKVGEGEPTTEKIT  
KQPVDEI  
VHYGGEQIPQGHKDEFDPNAPVDSKTEVPGKPGVKNPDTGEVVTTPVDD  
VT  
25 KYGPVDGDSITSTEEIPFDKKREFDPNLAPGTEKVVQKGEPGKTITTTPTTKN  
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30 ***S. epidermidis* aap DNA (SEQ ID NO:20)**

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5 **A-Domain from *S. epidermidis* Aap (amino acids 55-600) (SEQ ID NO:21)**

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 10 ANSDNDTQTQFSEAPTRNDLARKEDIPAVSKNEELQSSQPNTDSKIEPTTSEPVNL  
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 TFVKND SQGNTSKVGSGTPSTDFLNYADNTTNDLDGKFHGQKLNNVNLKYNASN  
 15 QTFTATYAGKTWTATLSELGLSPTDSYNFLVTSSQYGNGNSGTYASGVMRADLD  
 GA<sup>600</sup>

**Protein Production and Purification**

20 Using PCR, the A domain of DgsK or SasA was amplified from the sequences  
 described above and subcloned into the *E. coli* expression vector PQE-30 (Qiagen),  
 which allows for the expression of a recombinant fusion protein containing six  
 histidine residues. This vector was subsequently transformed into the *E. coli* strain  
 25 ATCC 55151, grown in a 15-liter fermentor to an optical density (OD<sub>600</sub>) of 0.7 and  
 induced with 0.2 mM isopropyl-1-beta-D galactoside (IPTG) for 4 hours. The cells  
 were harvested using an AG Technologies hollow-fiber assembly (pore size of 0.45  
 μm) and the cell paste frozen at -80° C. Cells were lysed in 1X PBS (10 mL of  
 buffer/1 g of cell paste) using 2 passes through the French Press @ 1100psi.  
 30 Lysed cells were spun down at 17,000rpm for 30 minutes to remove cell debris.  
 Supernatant was passed over a 5-mL HiTrap Chelating (Pharmacia) column  
 charged with 0.1M NiCl<sub>2</sub>. After loading, the column was washed with 5 column

volumes of 10mM Tris, pH 8.0, 100mM NaCl (Buffer A). Protein was eluted using a 0-100% gradient of 10mM Tris, pH 8.0, 100mM NaCl, 200 mM imidazole (Buffer B) over 30 column volumes. SdrGN1N2N3 or SdrGN2N3 eluted at ~13% Buffer B (~26mM imidazole). Absorbance at 280nm was monitored. Fractions containing  
5 SdrGN1N2N3 or SdrGN2N3 were dialyzed in 1x PBS.

Each protein was then put through an endotoxin removal protocol. Buffers used during this protocol were made endotoxin free by passing over a 5-mL Mono-Q sepharose (Pharmacia) column. Protein was divided evenly between 4x 15mL tubes. The volume of each tube was brought to 9mL with Buffer A. 1mL of 10%  
10 Triton X-114 was added to each tube and incubated with rotation for 1 hour at 4°C. Tubes were placed in a 37°C water bath to separate phases. Tubes were spun down at 2,000rpm for 10 minutes and the upper aqueous phase from each tube was collected and the detergent extraction repeated. Aqueous phases from the 2nd extraction were combined and passed over a 5-mL IDA chelating (Sigma) column,  
15 charged with 0.1M NiCl<sub>2</sub> to remove remaining detergent. The column was washed with 9 column volumes of Buffer A before the protein was eluted with 3 column volumes of Buffer B. The eluant was passed over a 5-mL Detoxigel (Sigma) column and the flow-through collected and reappplied to the column. The flow-through from the second pass was collected and dialyzed in 1x PBS. The purified  
20 product was analyzed for concentration, purity and endotoxin level before administration into the mice.

### **Monoclonal Antibody Production**

25 *E. coli* expressed and purified recombinant SasA and DsgK proteins were used to generate a panel of murine monoclonal antibodies while the mouse sera was used as a source of polyclonal antibodies. Briefly, a group of Balb/C or SJL mice received a series of subcutaneous immunizations of 1-10 mg of protein in solution or mixed with adjuvant as described in the Table below.

### **Immunization Schemes**

RIMMS				
Injection	Day	Amount (µg)	Route	Adjuvant
#1	0	5	Subcutaneous	FCA/RIBI
#2	2	1	Subcutaneous	FCA/RIBI
#3	4	1	Subcutaneous	FCA/RIBI
#4	7	1	Subcutaneous	FCA/RIBI
#5	9	1	Subcutaneous	FCA/RIBI

Conventional				
Injection	Day	Amount (µg)	Route	Adjuvant
Primary	0	5	Subcutaneous	FCA
Boost #1	14	1	Intraperitoneal	RIBI
Boost #2	28	1	Intraperitoneal	RIBI
Boost #3	42	1	Intraperitoneal	RIBI

At the time of sacrifice (RIMMS) or seven days after a boost (conventional) serum was collected and titred in ELISA assays against MSCRAMM<sup>®</sup> proteins or on whole cells (*S. epidermidis* and *S. aureus*). Three days after the final boost, the spleens or lymph nodes were removed, teased into a single cell suspension and the lymphocytes harvested. The lymphocytes were then fused to a P3X63Ag8.653 myeloma cell line (ATCC #CRL-1580). Cell fusion, subsequent plating and feeding were performed according to the Production of Monoclonal Antibodies protocol from Current Protocols in Immunology (Chapter 2, Unit 2.).

Any clones that were generated from the fusion were then screened for specific anti-SasA antibody production using a standard ELISA assay. Positive clones were expanded and tested further for activity in a whole bacterial cell binding assay by flow cytometry and SasA binding by Biacore analysis.

### Biacore Analysis

Throughout the analysis, the flow rate remained constant at 10 ml/min. Prior to the SasA or DgsK injection, test antibody was adsorbed to the chip via RAM-Fc binding. At time 0, SasA or DgsK at a concentration of 30 mg/ml was injected over the chip for 3 min followed by 2 minutes of dissociation. This phase of the analysis



measured the relative association and disassociation kinetics of the Mab / SasA or DgsK interaction.

### Binding to Whole Bacteria

5

Bacterial samples *S. aureus* Newman, *S. aureus* 67-0, *S. aureus* 397 (Sal6), *S. aureus* Wood, *S. aureus* 8325-4, methicillin resistant *S. aureus* MRSA 16, *S. epidermidis* ATCC 35984, *S. epidermidis* HB, *S. epidermidis* CN-899 and *S. haemolyticus* ATCC 43253 were collected, washed and incubated with Mab or PBS alone (control) at a concentration of 2 µg/ml after blocking with rabbit IgG (50 mg/ml). Following incubation with antibody, bacterial cells were incubated with Goat-F<sub>(ab)</sub><sup>2</sup>-Anti-Mouse-F<sub>(ab)</sub><sup>2</sup>-FITC which served as the detection antibody. After antibody labeling, bacterial cells were aspirated through the FACScaliber flow cytometer to analyze fluorescence emission (excitation: 488, emission: 570). For each bacterial strain, 10,000 events were collected and measured. These data indicate that antibodies against *S. aureus* SasA were able to recognize a homologous protein on the surface of coagulase-negative staphylococci. The data support Western blot analysis demonstrating that rabbit polyclonal antibodies against *S. aureus* SasA cross-react with a protein released from the cell surface of *S. epidermidis* HB as well as the recombinant A-region from DsgK cloned from *S. epidermidis* (see Table below and Figure 6).

20

### Polyclonal Sera Reactivity

	Newman	67-0	397 (Sal6)	Wood 46	8325-4	MRSA 16	ATCC 35984	HB	CN-899	ATCC 43253
Normal Mouse Sera	-	-	-	-	-	-	-	-	-	-
Mouse anti-SasA	+	+	+/-	-	+	+	+	+	+	+

What is claimed is:

1. An isolated antibody which binds to a staphylococcal surface protein selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17,  
5 18, 19 and 21.

2. The antibody according to Claim 1 wherein the antibody is raised against the A domain of the surface protein.

10 3. The antibody according to Claim 1, wherein the antibody treats or prevents *S. aureus* infection in a human or animal.

4. The antibody according to Claim 1, wherein the antibody is suitable for parenteral, oral, intranasal, subcutaneous, aerosolized or intravenous administration  
15 in a human or animal.

5. The antibody according to Claim 1, wherein said antibody is a monoclonal antibody.

20 6. The antibody according to Claim 1, wherein said antibody is a polyclonal antibody.

7. The antibody according to Claim 5 wherein the monoclonal antibody is of a type selected from the group consisting of murine, chimeric, humanized and  
25 human monoclonal antibodies.

8. The antibody according to Claim 5 wherein the antibody is a single chain monoclonal antibody.

9. The antibody according to Claim 1 which comprises an antibody fragment having the same binding specificity of an antibody which binds to a staphylococcal surface protein having the sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21.

10. The antibody according to Claim 1 that is raised against a protein having an amino acid sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21.

11. The antibody according to Claim 1 wherein the surface protein has an amino acid sequence encoded by a nucleic acid sequence selected from the group consisting of nucleic acid sequences SEQ ID NOS. 1, 3, 5, 7, 9, 11, 13, 15, 20 and the nucleic acid sequences coding for the A domain of the Aap protein or degenerates thereof.

12. Isolated antisera containing an antibody according to Claim 1.

13. A diagnostic kit comprising an antibody according to Claim 1 and means for detecting binding by that antibody.

14. A diagnostic kit according to Claim 13 wherein said means for detecting binding comprises a detectable label that is linked to said antibody.

15. A method of diagnosing an infection of *S. aureus* comprising adding an antibody according to Claim 1 to a sample suspected of being infected with *S. aureus*, and determining if antibodies have bound to the sample.

16. A pharmaceutical composition for treating or preventing an infection of *S. aureus* comprising an effective amount of the antibody of Claim 1 and a pharmaceutically acceptable vehicle, carrier or excipient.

5 17. A method of treating or preventing an infection of *S. aureus* comprising administering to a human or animal patient an effective amount of an antibody according to Claim 1.

10 18. A method of inducing an immunological response comprising administering to a human or animal an immunogenic amount of an isolated protein selected from the group consisting of the amino acid sequences SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21.

15 19. An isolated antibody according to Claim 1 that has the ability to bind to an amino acid sequence coded by the nucleic acid sequence of SEQ ID NOS. 1, 3, 5, 7, 9, 11, 13, 15, 20 and the nucleic acid sequences coding for the A domain of the Aap protein or degenerates thereof.

20 20. An isolated active fragment from the A domain of the DsqA protein.

21. An isolated antibody according to Claim 1 further comprising a physiologically acceptable antibiotic.

25 22. A vaccine for treating or preventing an infection of *S. aureus* comprising an amount of a protein sequence selected from the group consisting of SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 17, 18, 19 and 21 in an amount effective to elicit an immune response, and a pharmaceutically acceptable vehicle, carrier or excipient.

Figure 1. Primary structure of in silico-predicted LPXTG proteins.

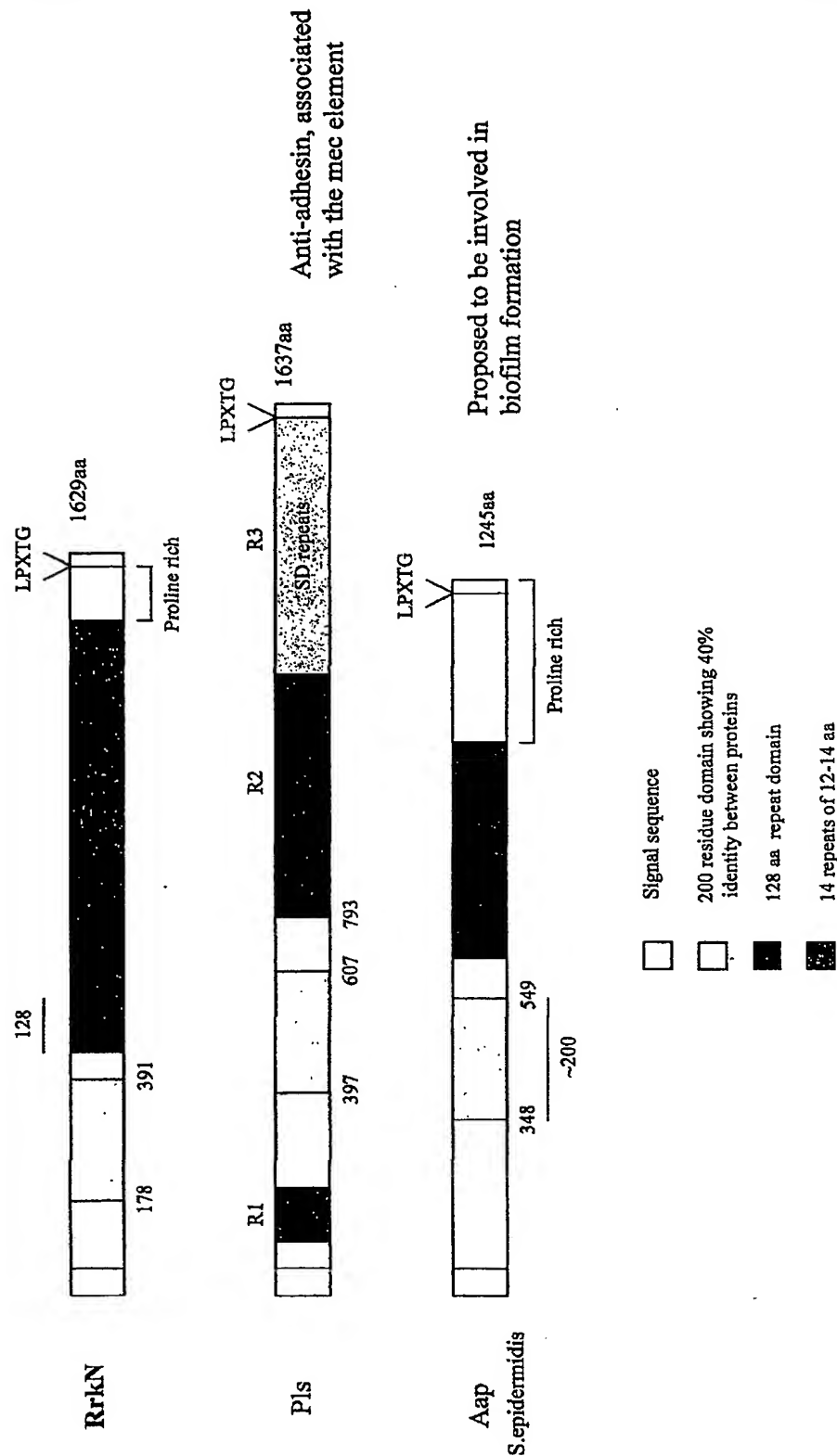
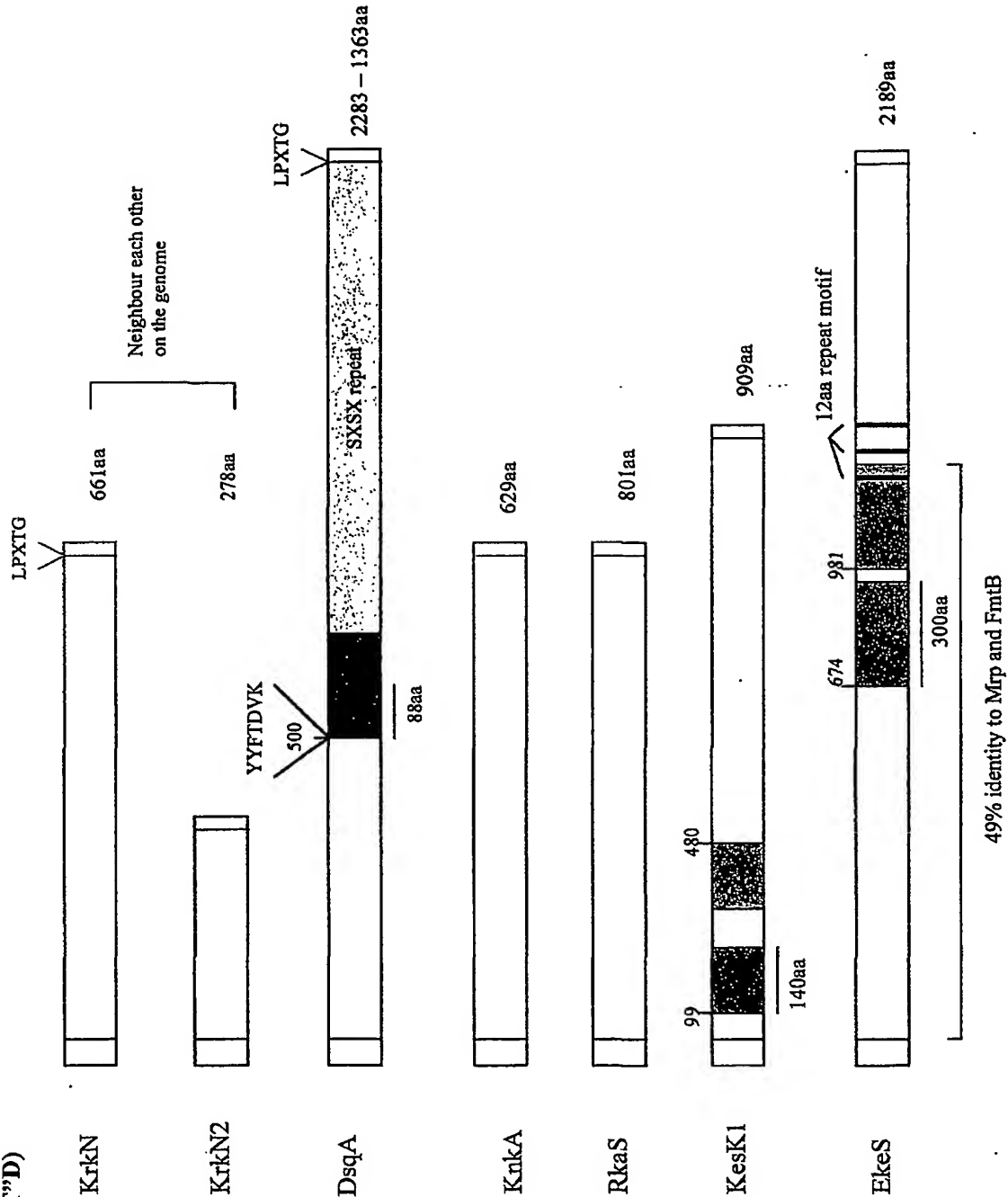
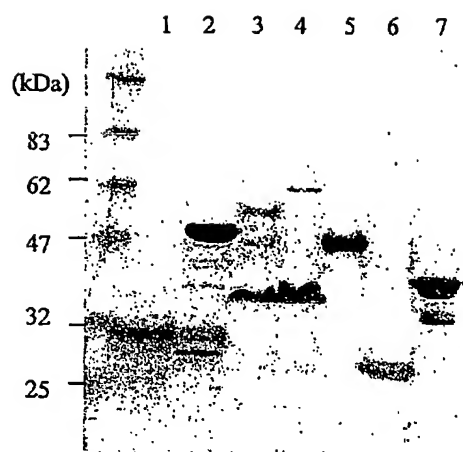


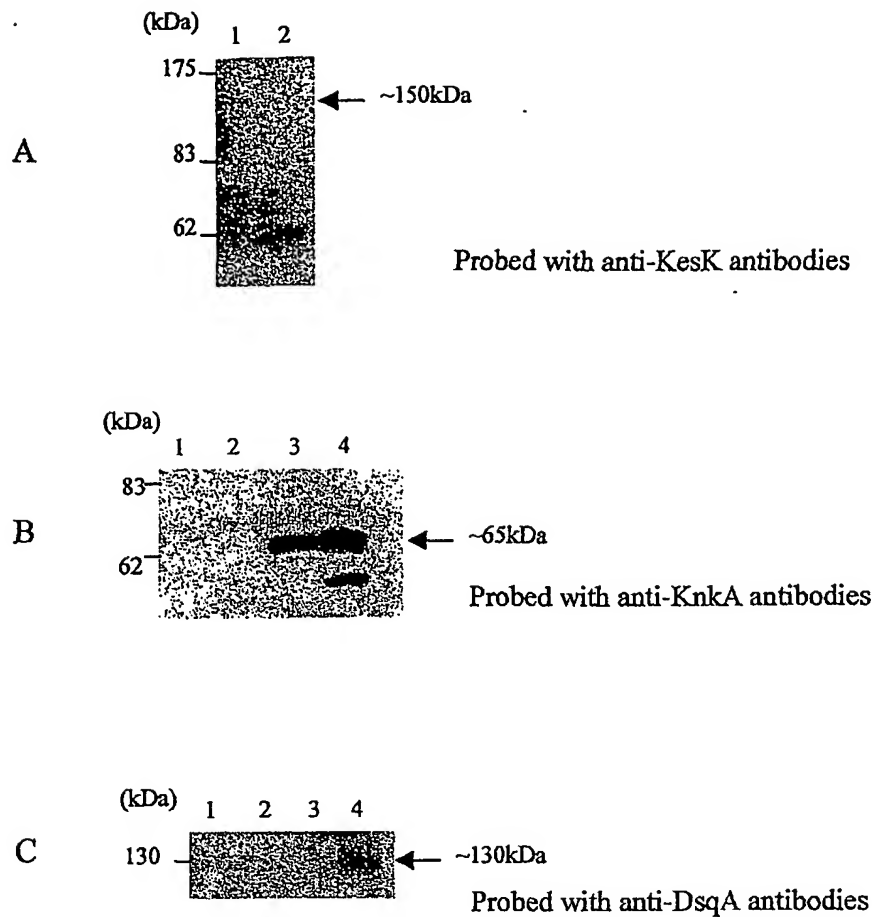
Figure 1 (CONT'D)





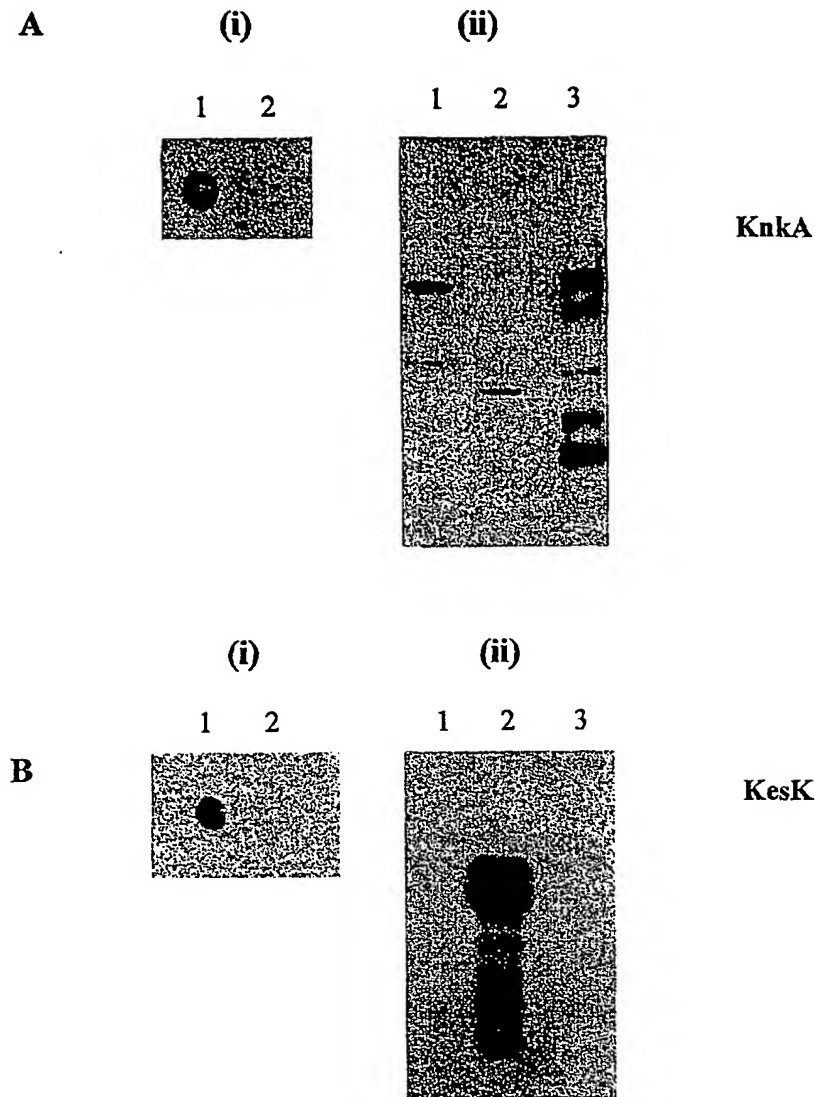
	Residues	Predicted MW	Apparent MW
• RrkN 1	60 - 215	19	29
• RrkN 2	60 - 437	45	48
• DsqA 1	54 - 279	27	38
• DsqA 2	54 - 533	58	62
• KesK 1	55 - 335	34	47
• KnkA	39 - 210	20	27
• KesK 2	329 - 591	31	40

**Figure 2. Coomassie gel of the purified N-terminal His-tagged fusion proteins.**



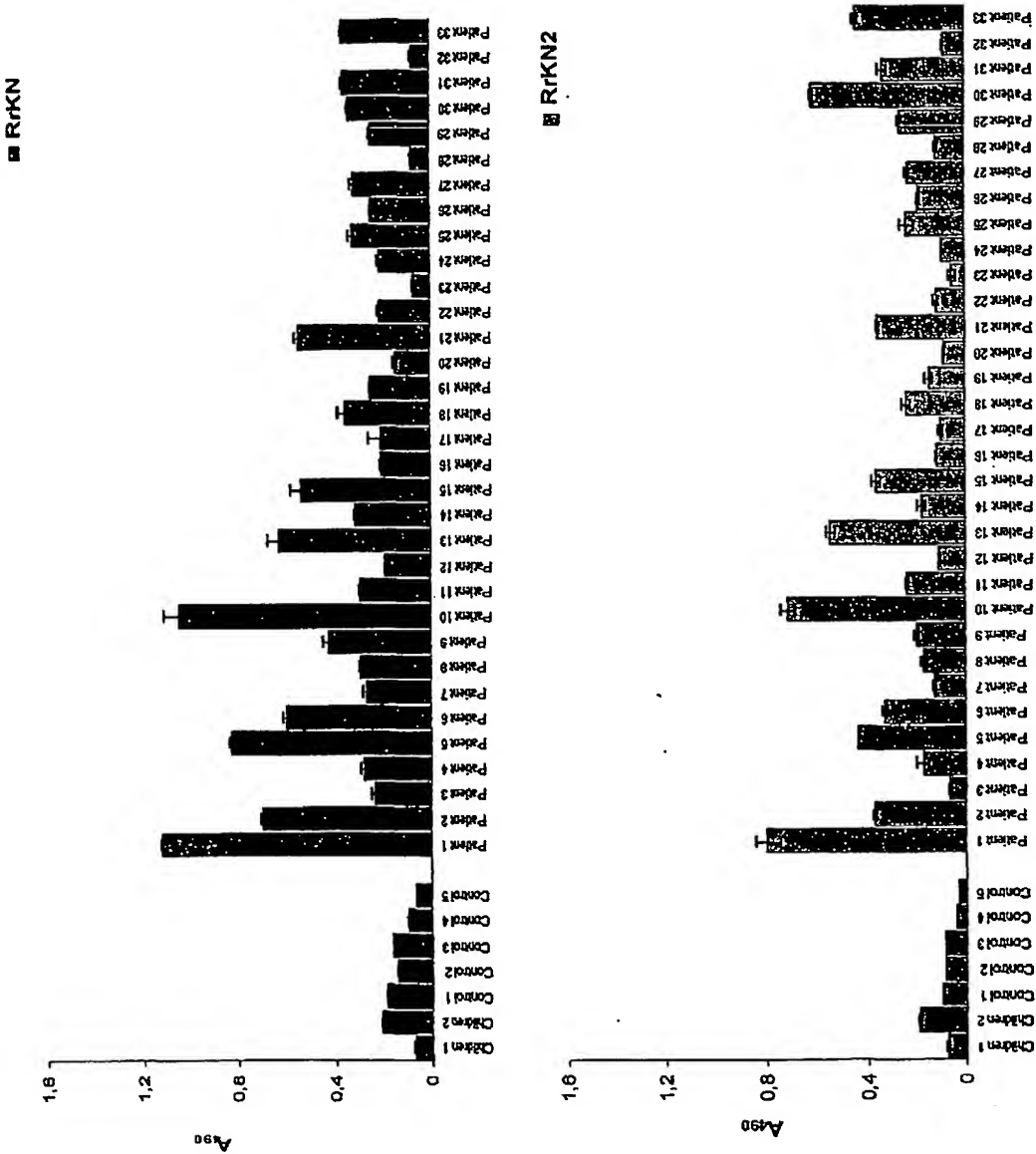
**Figure 3. Western blotting of *S.aureus* cell wall extracts.** Bacterial cells were standardised to an OD<sub>600</sub> of 50 units and cell walls were isolated by lysostaphin digestion of stabilised protoplasts.  
A. Lane 1, 8325-4 (early exponential phase); lane 2, 8325-4 (stationary phase).  
B. Lanes 1 and 2, eMRSA-16 ; lanes 3 and 4, 8325-4; lanes 1 and 3 represent early exponential phase cells and lanes 2 and 4 represent stationary phase cells.  
C. Lanes 1 and 2, MSSA ; lanes 3 and 4, eMRSA-16; lanes 1 and 3 represent early exponential phase cells and lanes 2 and 4 represent stationary phase cells.



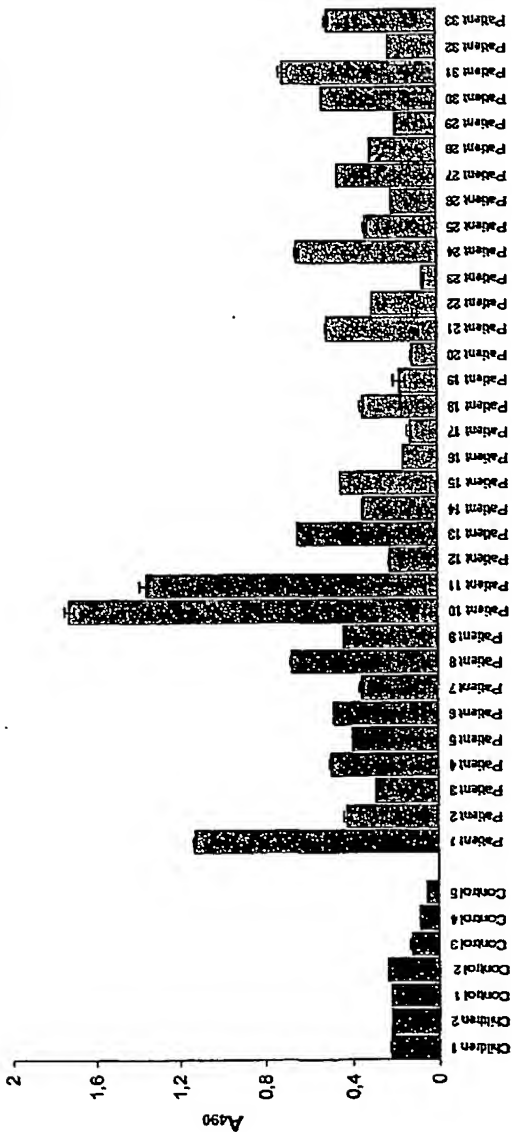


**Figure 4. Dot blotting and Western immunoblotting of *Lactococcus lactis* expressing *S.aureus* MSCRAMMs.** Full length *knkA* and *kesK* were cloned into the *L.lactis* expression plasmid pKS80 and electroporated into competent *L.lactis* MG1363 cells. Positive KnkA and KesK expressing clones were detected using dot blotting with anti-KnkA (A) and anti-KesK (B) antibodies, respectively. *L.lactis* bearing pKS80 was used as a negative control. A.(i) lane 1, *L.lactis* pKS-KnkA; lane 2, *L.lactis* pKS80. B. (ii) lane 1, *L.lactis* pKS-KesK; lane 2, *L.lactis* pKS80. Western immunoblotting was used to examine the expression of KesK and KnkA in *S.aureus* and *L.lactis*. A (ii). Lane 1, cell wall extract from exponential phase *S.aureus* strain 8325-4, lane 2, protoplast fraction from *L.lactis* bearing pKS80; lane 3, protoplast fraction from *L.lactis* bearing pKS-KnkA. B. (ii) Lane 1, cell wall extract from exponential phase *S.aureus* strain 8325-4; lane 2, cell wall extract from *L.lactis* bearing pKS-KesK; lane 3, cell wall extract from *L.lactis* bearing pKS80.

Figure 5A. Probing recombinant LPXTG proteins with convalescent sera to study *in vivo* expression.



▣ Kesk1



▣ Kesk2A

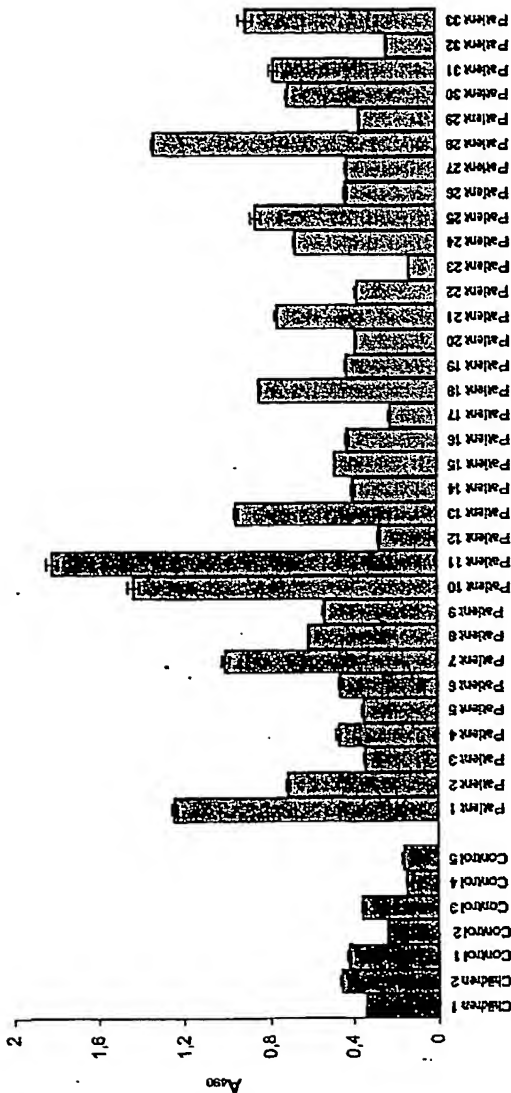
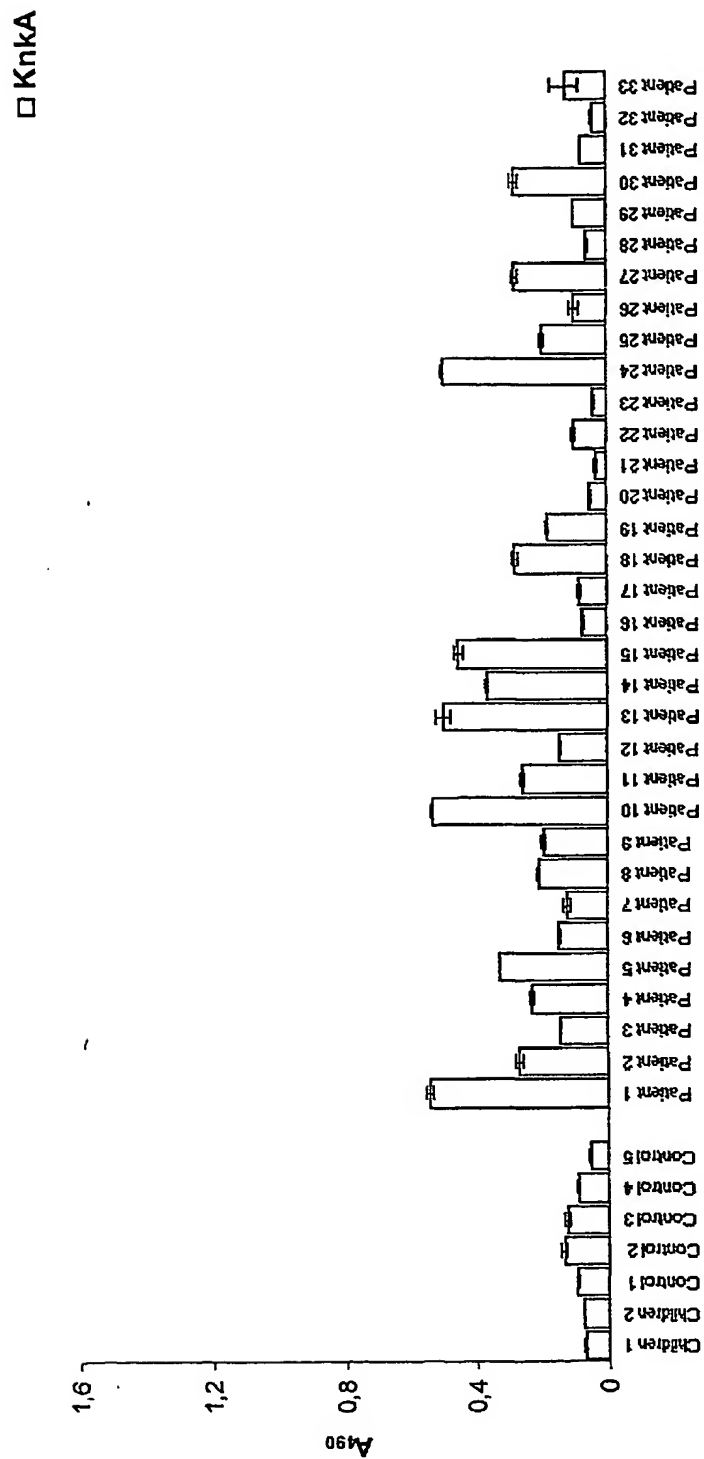


Figure 5B

Figure 5C



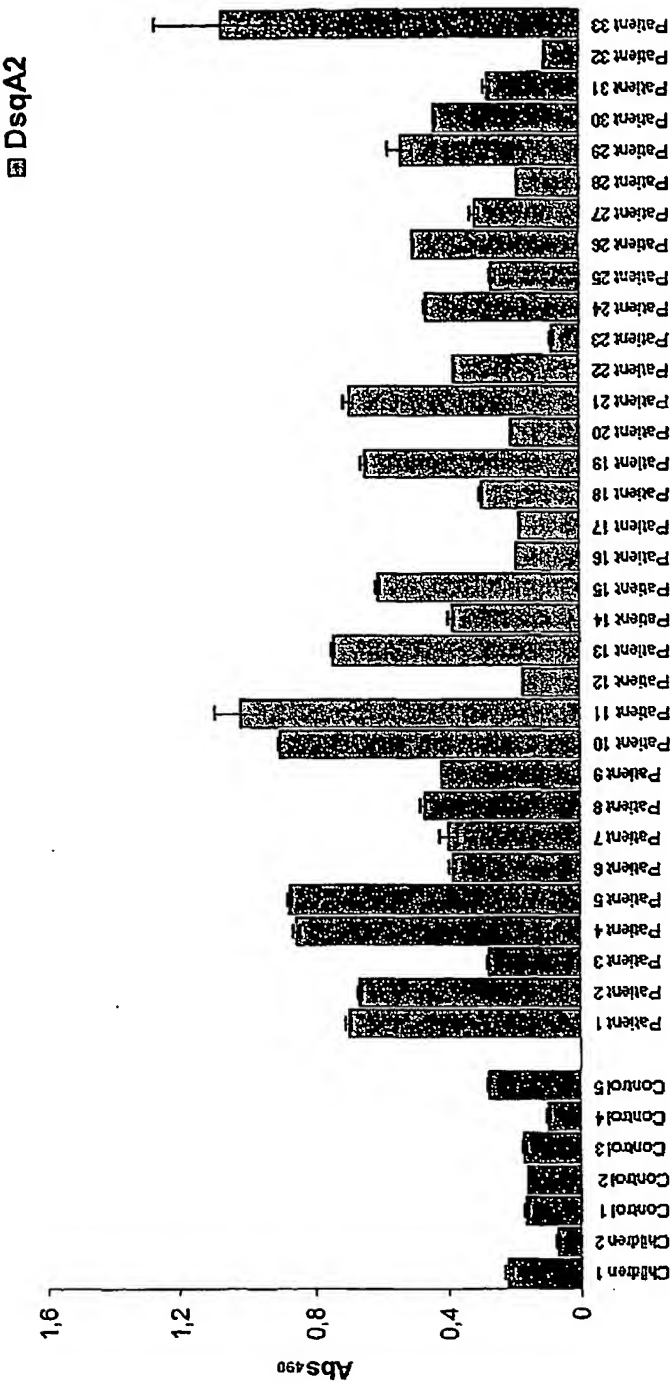
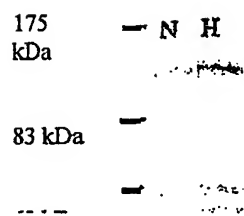
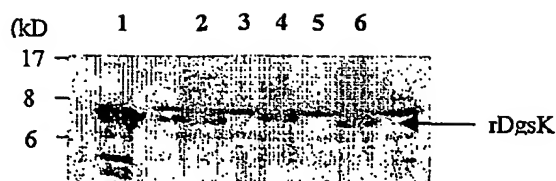


Figure 5D



Western immunoblotting analysis of proteins released from the cell wall of *S. aureus* Newman (N) and *S. epidermidis* HB (H). Probed with rabbit anti-*S. aureus* SasA region A antibodies and goat anti-rabbit conjugated to horseradish peroxidase



Cross reaction of *S. aureus* SasA A-region antibodies with DgsK expressed in *E. coli*. Lane 1, FPLC purified SasA A-region control. Lanes 2, 4 and 6, DgsK A-region expressed from pQE-30 in *E. coli* strain TOPP-3 (induced); lanes 3, 5 and 7, TOPP-3 bearing pQE-30 with *dgsK* insert (uninduced).

**FIGURE 6**

## SEQUENCE LISTING

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 <120> CROSS-REACTIVE MONOCLONAL AND POLYCLONAL ANTIBODIES. . .  
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Asn Gly Ala Gln Ala Leu Thr Thr Asp His Asn Val Gln Gly Gly Ser  
 35 40 45

20 Asn Gln Ala Leu Pro Gly Asn Ser Gln Asn Thr Asn Ala Asp Thr Asn  
 50 55 60

25 Arg Asp Ile Val Asn Asp Ser Gln Asn Thr Pro Asn Ala His Ala Thr  
 65 70 75 80

Asp Asn Thr Ser Thr Asn Gln Ala Leu Thr Asn His Gln Asn Val Asp  
 85 90 95

30 Val Ala Asn Gln Val Gly Pro Ala Pro Ile Gln Pro Ser Ala Ser Pro  
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Lys Glu Ile Gln Glu Asp Val Arg His Ser Ser Asp Lys Pro Glu Leu  
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50 Thr Pro Ala Asp Pro Thr Ala Gly Asn Gly Ser Ala Pro Val Ala Ile  
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	Ile Asn Ser Asn Arg Glu Ala Thr Gln Glu Glu Lys Asn Ala Ala Leu				
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15	Glu Pro His Ile Val Val	Lys Pro Glu Ala Gln	Glu Ala Ile Lys		
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 ggtggtgaac aaataccaca aggtcataaa gatgaatttg atccaaatgc acctgtagat 2880  
 agtaaaactg aagttccagg taaaccagga gttaaaaatc ctgatacagg tgaagttgtt 2940  
 35 accccaccag tggatgatgt gacaaaatat ggtccagttg atggagattc gattacgtca 3000  
 acggaagaaa ttccgtttga taaaaaacgc gaatttgatc caaacttagc gccagggtaca 3060  
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 40 aaccattaa caggagaaaa agttggcgaa ggtaaatcaa cagaaaaagt cactaaacaa 3180  
 cctgttgacg aaattgttga gtatggtcca acaaaagcag aaccaggtaa accagcgaa 3240  
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 50 gcggaaccag gtaaacagc ggaaccaggt aaaccagcgg aaccaggtac gccagcagaa 3420  
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 tcaacagata ataaaatca attacctgat acaggtgaaa atcgtcaagc taatgagga 3660  
 60 actttagtcg gatctctatt agcaattgtc ggatcattgt tcatatttg tcgtcgtaaa 3720



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3765

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 <211> 546  
 <212> PRT  
 <213> Staphylococcus epidermidis  
  
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 Glu Lys Gln Val Asp Pro Ile Thr Gln Ala Asn Gln Asn Asp Ser Ser  
 1 5 10 15  
 15 Glu Arg Ser Leu Glu Asn Thr Asn Gln Pro Thr Val Asn Asn Glu Ala  
 20 25 30  
 Pro Gln Met Ser Ser Thr Leu Gln Ala Glu Glu Gly Ser Asn Ala Glu  
 35 40 45  
 20 Ala Pro Gln Ser Glu Pro Thr Lys Ala Glu Glu Gly Gly Asn Ala Glu  
 50 55 60  
 25 Ala Ala Gln Ser Glu Pro Thr Lys Ala Glu Glu Gly Gly Asn Ala Glu  
 65 70 75 80  
 30 Ala Pro Gln Ser Glu Pro Thr Lys Ala Glu Glu Gly Gly Asn Ala Glu  
 85 90 95  
 Ala Ala Gln Ser Glu Pro Thr Lys Thr Glu Glu Gly Ser Asn Val Lys  
 100 105 110  
 Ala Ala Gln Ser Glu Pro Thr Lys Ala Glu Glu Gly Ser Asn Ala Glu  
 115 120 125  
 35 Ala Pro Gln Ser Glu Pro Thr Lys Thr Glu Glu Gly Ser Asn Ala Lys  
 130 135 140  
 Ala Ala Gln Ser Glu Pro Thr Lys Ala Glu Glu Gly Gly Asn Ala Glu  
 145 150 155 160  
 40 Ala Ala Gln Ser Glu Pro Thr Lys Thr Glu Glu Gly Ser Asn Ala Glu  
 165 170 175  
 45 Ala Pro Gln Ser Glu Pro Thr Lys Ala Glu Glu Gly Gly Asn Ala Glu  
 180 185 190  
 Ala Pro Gln Ser Glu Pro Thr Lys Thr Glu Glu Gly Gly Asn Ala Glu  
 195 200 205  
 50 Ala Pro Asn Val Pro Thr Ile Lys Ala Asn Ser Asp Asn Asp Thr Gln  
 210 215 220  
 Thr Gln Phe Ser Glu Ala Pro Thr Arg Asn Asp Leu Ala Arg Lys Glu  
 225 230 235 240  
 55 Asp Ile Pro Ala Val Ser Lys Asn Glu Glu Leu Gln Ser Ser Gln Pro  
 245 250 255  
 60 Asn Thr Asp Ser Lys Ile Glu Pro Thr Thr Ser Glu Pro Val Asn Leu  
 260 265 270

Asn Tyr Ser Ser Pro Phe Met Ser Leu Leu Ser Met Pro Ala Asp Ser  
 275 280 285  
 5 Ser Ser Asn Asn Thr Lys Asn Thr Ile Asp Ile Pro Pro Thr Thr Val  
 290 295 300  
 Lys Gly Arg Asp Asn Tyr Asp Phe Tyr Gly Arg Val Asp Ile Glu Ser  
 305 310 315 320  
 10 Asn Pro Thr Asp Leu Asn Ala Thr Asn Leu Thr Arg Tyr Asn Tyr Gly  
 325 330 335  
 Gln Pro Pro Gly Thr Thr Thr Ala Gly Ala Val Gln Phe Lys Asn Gln  
 340 345 350  
 15 Val Ser Phe Asp Lys Asp Phe Asp Phe Asn Ile Arg Val Ala Asn Asn  
 355 360 365  
 Arg Gln Ser Asn Thr Thr Gly Ala Asp Gly Trp Gly Phe Met Phe Ser  
 370 375 380  
 Lys Lys Asp Gly Asp Asp Phe Leu Lys Asn Gly Gly Ile Leu Arg Glu  
 385 390 395 400  
 25 Lys Gly Thr Pro Ser Ala Ala Gly Phe Arg Ile Asp Thr Gly Tyr Tyr  
 405 410 415  
 Asn Asn Asp Pro Leu Asp Lys Ile Gln Lys Gln Ala Gly Gln Gly Tyr  
 420 425 430  
 Arg Gly Tyr Gly Thr Phe Val Lys Asn Asp Ser Gln Gly Asn Thr Ser  
 435 440 445  
 35 Lys Val Gly Ser Gly Thr Pro Ser Thr Asp Phe Leu Asn Tyr Ala Asp  
 450 455 460  
 Asn Thr Thr Asn Asp Leu Asp Gly Lys Phe His Gly Gln Lys Leu Asn  
 465 470 475 480  
 40 Asn Val Asn Leu Lys Tyr Asn Ala Ser Asn Gln Thr Phe Thr Ala Thr  
 485 490 495  
 Tyr Ala Gly Lys Thr Trp Thr Ala Thr Leu Ser Glu Leu Gly Leu Ser  
 500 505 510  
 Pro Thr Asp Ser Tyr Asn Phe Leu Val Thr Ser Ser Gln Tyr Gly Asn  
 515 520 525  
 50 Gly Asn Ser Gly Thr Tyr Ala Ser Gly Val Met Arg Ala Asp Leu Asp  
 530 535 540  
 Gly Ala  
 545  
 55 <210> 22  
 <211> 36  
 <212> PRT  
 <213> Staphylococcus aureus  
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&lt;400&gt; 22

Leu Pro Asn Thr Gly Ser Glu Glu Met Asp Leu Pro Leu Lys Glu Leu  
 1 5 10 15

Ala Leu Ile Thr Gly Ala Ala Leu Leu Ala Arg Arg Arg Ser Lys Lys  
 20 25 30

Glu Lys Glu Ser  
 35

&lt;210&gt; 23

&lt;211&gt; 43

&lt;212&gt; PRT

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 23

Leu Pro Asp Thr Gly Asp Ser Ile Lys Gln Asn Gly Leu Leu Gly Gly  
 1 5 10 15

Val Met Thr Leu Leu Val Gly Leu Gly Leu Met Lys Arg Lys Lys Lys  
 20 25 30

Lys Asp Glu Asn Asp Gln Asp Asp Ser Gln Ala  
 35 40

&lt;210&gt; 24

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 24

Leu Pro Lys Thr Gly Glu Thr Thr Ser Ser Gln Ser Trp Trp Gly Leu  
 1 5 10 15

Tyr Ala Leu Leu Gly Met Leu Ala Leu Phe Ile Pro Lys Phe Arg Lys  
 20 25 30

Glu Ser Lys  
 35

&lt;210&gt; 25

&lt;211&gt; 38

&lt;212&gt; PRT

&lt;213&gt; Staphylococcus aureus

&lt;400&gt; 25

Leu Pro Lys Thr Gly Leu Thr Ser Val Asp Asn Phe Ile Ser Thr Val  
 1 5 10 15

Ala Phe Ala Thr Leu Ala Leu Leu Gly Ser Leu Ser Leu Leu Leu Phe  
 20 25 30

Lys Arg Lys Glu Ser Lys  
 35

&lt;210&gt; 26

<211> 36  
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 <213> Staphylococcus aureus

5 <400> 26

Leu Pro Gln Thr Gly Glu Glu Ser Asn Lys Asp Met Thr Leu Pro Leu  
 1 5 10 15

10 Met Ala Leu Ile Ala Leu Ser Ser Ile Val Ala Phe Val Leu Pro Arg  
 20 25 30

Lys Arg Lys Asn  
 35

15

<210> 27  
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<400> 27

Leu Pro Lys Thr Gly Thr Asn Gln Ser Ser Ser Pro Glu Ala Met Phe  
 1 5 10 15

25

Val Leu Leu Ala Gly Ile Gly Leu Ile Ala Thr Val Arg Arg Arg Lys  
 20 25 30

Ala Ser

30

<210> 28  
 <211> 33  
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35

<400> 28

Leu Pro Lys Thr Gly Leu Glu Ser Thr Gln Lys Gly Leu Ile Phe Ser  
 1 5 10 15

40

Ser Ile Ile Gly Ile Ala Gly Leu Met Leu Leu Ala Arg Arg Arg Lys  
 20 25 30

Asn

45

<210> 29  
 <211> 39  
 <212> PRT  
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50

<400> 29

Leu Pro Lys Ala Gly Glu Thr Ile Lys Glu His Trp Leu Pro Ile Ser  
 1 5 10 15

55

Val Ile Val Gly Ala Met Gly Val Leu Met Ile Trp Leu Ser Arg Arg  
 20 25 30

Asn Lys Leu Lys Asn Lys Ala  
 35

60